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ISSN 2039-0394 (Print Edition)
ISSN 2039-0408 (Online Edition)

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Biodiversity Journal

DECEMBER 2019, 10 (4): 305-608

FOR WILDLIFE RESEARCH
AND ENVIRONMENTAL STUDIES

Muscari gussonei (Parl.) Nyman - Italy, Sicily (CL): Gela, Poggio Arena

BIODIVERSITY JOURNAL
2019,10 (4): 305-608

Quaternly scientific journal
edited by Edizioni Danaus,
via V. Di Marco 43, 90143 Palermo, Italy
www.biodiversityjournal.com
biodiversityjournal@gmail.com

Official authorization no. 40 (28.12.2010)

ISSN 2039-0394 (Print Edition)
ISSN 2039-0408 (Online Edition)

***Muscari gussonei* (Parl.) Nyman**. It is an endemic plant of the Asparagaceae family that lives exclusively in Sicily. It grows solely on sandy soils and on consolidated dune systems, preferring the area behind the dunes, which is less exposed to winds. It is distributed in the south-eastern coast of Sicily from Gela to Vittoria with a highly fragmented range and with a spring bloom. In the last decades, this species has progressively shrunk due to lack of land management policies and the rarefaction of its habitat threatened by greenhouse cultivation and excessive coastal urbanization. *Muscari gussonei* is protected by the Habitats Directive 92/43/EEC (Annex II), by the Berne Convention (Annex I) and is included in the IUCN Red List as EN, for Endangered. During the 2012-2016 period, the project LIFE11 NAT/IT/000232 "Dune habitats protection in the greenhouse landscape of the Gela Gulf for the safeguarding of *Leopoldia gussonei*" was carried out for the conservation of this species. With this project, targeted interventions were carried out in order to identify and monitor populations, decrease anthropic pressure on dune habitats, restore and improve the management of degraded dune and behind the dunes environments, encourage agricultural systems with low environmental impact, increase connectivity to internal ecological network in protected areas and increase information and encourage the didactic use of dune areas compatible with the conservation needs of the species. The beneficiaries involved in the LIFE project were the Department of Agriculture of the University of Catania, LIPU BirdLife Italia as the managing body of the RNO Biviere di Gela and the Rural and Territorial Development Department of the Sicily Region.

Amedeo Falci. Via Libertà 200, 93100 Caltanissetta, Italy; e-mail: gegenes@gmail.com (photos Amedeo Falci)

Above. *Muscari gussonei* - Italy, Sicily: Biviere di Gela (CL). Below. *Rhodanthidium sticticum* (Fabricius, 1787) on *Muscari gussonei*, Gela, Contrada Mignechi.

Biodiversity Journal

DECEMBER 2019, 10 (4): 305–608

Biodiversity Journal, 2019, 10 (4): 307–314 https://doi.org/10.31396/Biodiv.Jour.2019.10.4.307.314

Distribution and morphological diversity of *Astyanax rivularis* Lütken, 1874 (Teleostei Characiformes) in the upper São Francisco River basin, Brazil

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ABSTRACT *Astyanax* S.F. Baird et Girard, 1854 (Teleostei Characiformes) is one of the most well characterized of the neotropical ichthyofauna and is composed of fish with great ability to adapt to different environmental conditions and a wide spectrum of interaction in fish assemblages due to its structure and population density. This study presents the geographical distribution and morphological diversity of *Astyanax rivularis* Lütken, 1874, a fish historically complex and extremely diverse, in tributary streams of the left side of upper São Francisco River.

KEY WORDS Brazil; Neotropical Fish; Brazilian Savannah; Characidae.

Received 13.07.2019; accepted 22.10.2019; published online 28.10.2019

INTRODUCTION

Although the genus *Astyanax* Baird et Girard, 1854 is one of the most well characterized of the neotropical ichthyofauna, perhaps for its easy capture, certain biological characteristics of this fish indicate that further studies are necessary for better understanding its natural history. The formation of structured populations, combined with evolutionary processes, such as vicariance, certainly plays a key role in the diversity of the group, which emerges as a genus where allopatric speciation has probably occurred. However, it is very difficult to determine specific limits in the group. There are at least four groups of cryptic species proposed in the genus, where different taxa with diagnostic characters (usually chromosomal) are observed sharing the same specific denomination. These cases involve both morphological characteristics and chromosomal or molecular aspects.

The genus *Astyanax* is composed of fish with great ability to adapt to different environmental conditions and a wide spectrum of interaction in fish assemblages due to its structure and population density (Orsi et al., 2004). This is demonstrated by the wide geographical distribution of the group, which covers almost the entire neotropical region (Eigenmann, 1921) and comprises about 150 species distributed from southern United States to northern Argentina (Eschmeyer & Fong, 2019).

Garutti (1995) states that the genus *Astyanax* has a structured distribution, which suggests a high level of endemism: even within a single basin, there are multiple forms with relatively restricted geographic distribution. Therefore, it is assumed that *Astyanax* do not form a uniform group, but present variations between populations, probably because this group inhabits many different microenvironments (Garutti & Britski, 2000). However, they are more successful in the best preserved habitats (Orsi

et al., 2004). In fact, it is observed that populations of *Astyanax* present genetic structure along a river (Moysés & Almeida-Toledo, 2002; Prioli et al., 2002; Leuzzi et al., 2004; Paiva et al., 2006).

Astyanax rivularis (Fig. 1) was described by Lütken (1874) as *Tetragonopterus rivularis* from the collections of Reinhardt of the mid-nineteenth century in the Velhas River, a major tributary of the São Francisco River. Later, it was considered a subspecies of *A. scabripinnis* Jenyns, 1842: *A. scabripinnis rivularis* (Eigenmnann, 1921). Chromosomal and morphometric characteristics led Moreira-Filho & Bertollo (1989) to conclude that *A. scabripinnis* is a species complex of which about 14 are currently recognized (Bertaco & Lucena, 2006). According to the "Catalog of Fishes" (Eschmeyer & Fong, 2019), *A. rivularis* Lütken, 1874 (Characidae Stethaprioninae) is currently accepted as a valid species with distribution in São Francisco River basin.

Figure 1. Sample of *Astyanax rivularis* (80 mm standard length).

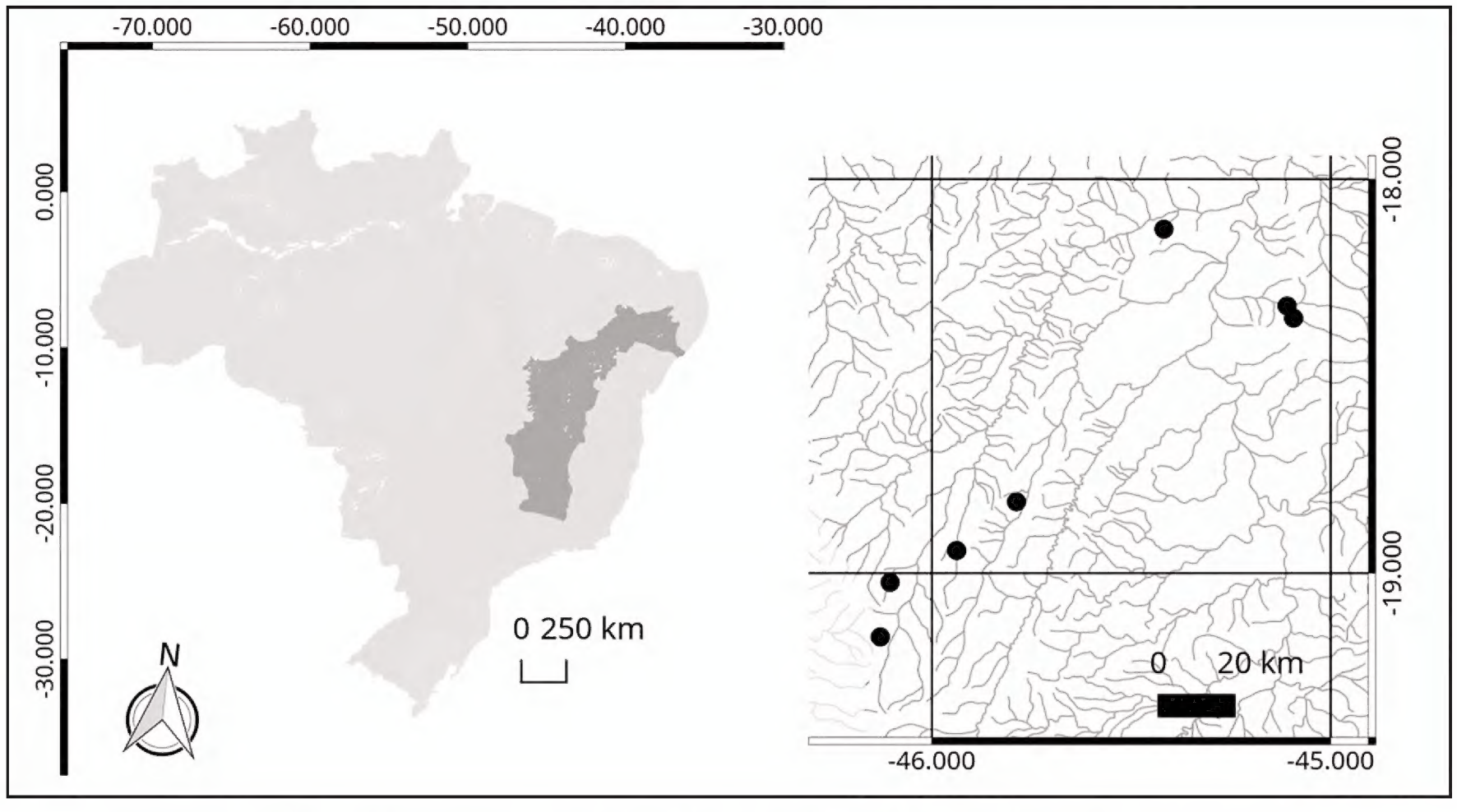

Figure 2. Map of Brazilian hydrographic basins with São Francisco river basin in dark gray (left); distribution of the samples in left tributaries of the Upper São Francisco river basin (right).

Here we present the morphometric data of seven populations of *A. rivularis*, with the purpose to better understand the distribution and diversity of the species.

MATERIAL AND METHODS

Seven population of *A. rivularis* were sampled totaling 69 individuals collected in tributaries of the left margin of Upper São Francisco River basin (Fig. 2), increasing the range of distribution of this species to Upper São Francisco River basin. Sampling and euthanizing of specimens were carried out in accordance with the recommendations of the Conselho Nacional de Controle de Experimentação Animal (CONCEA).

The specimens were deposited in the Collection of Vertebrates of the UFV Laboratory of Ecological and Evolutionary Genetics, at Rio Parnaíba Campus - CV-LaGEEvo. The identification was made according to Britski et al. (1988) and Lütken's description. The morphometric characters were obtained in millimeters (mm) with the help of a digital caliper with a 0.01 mm resolution.

All the data were obtained only from the left size of the sample considering the following measurements: standard head length, predorsal distance, prepelvic distance, preanal distance, height of dorsal origin, height of tail peduncle, length of anal basis, length of dorsal basis, length of pelvic basis, length of pectoral basis, head height, snout length, eye diameter, interorbital distance and jaw length.

RESULTS

All analyzed individuals were identified as *A. rivularis* according to available literature.

The standard length was between 41.44 cm to 82.73 cm (Tables 1 to 7). The highest standard length mean was found in Borrachudo stream population (70.36 cm), and the lowest was in Curral das Éguas stream (44.94 cm). The snout length and head height were the most variable characteristics, meanwhile the length of pectoral basis and the length of head were the less ones (Fig. 3). The scales in the lateral line range from 32 to 39, and the branched anal-fin rays range from 16 to 24 (see the tables).

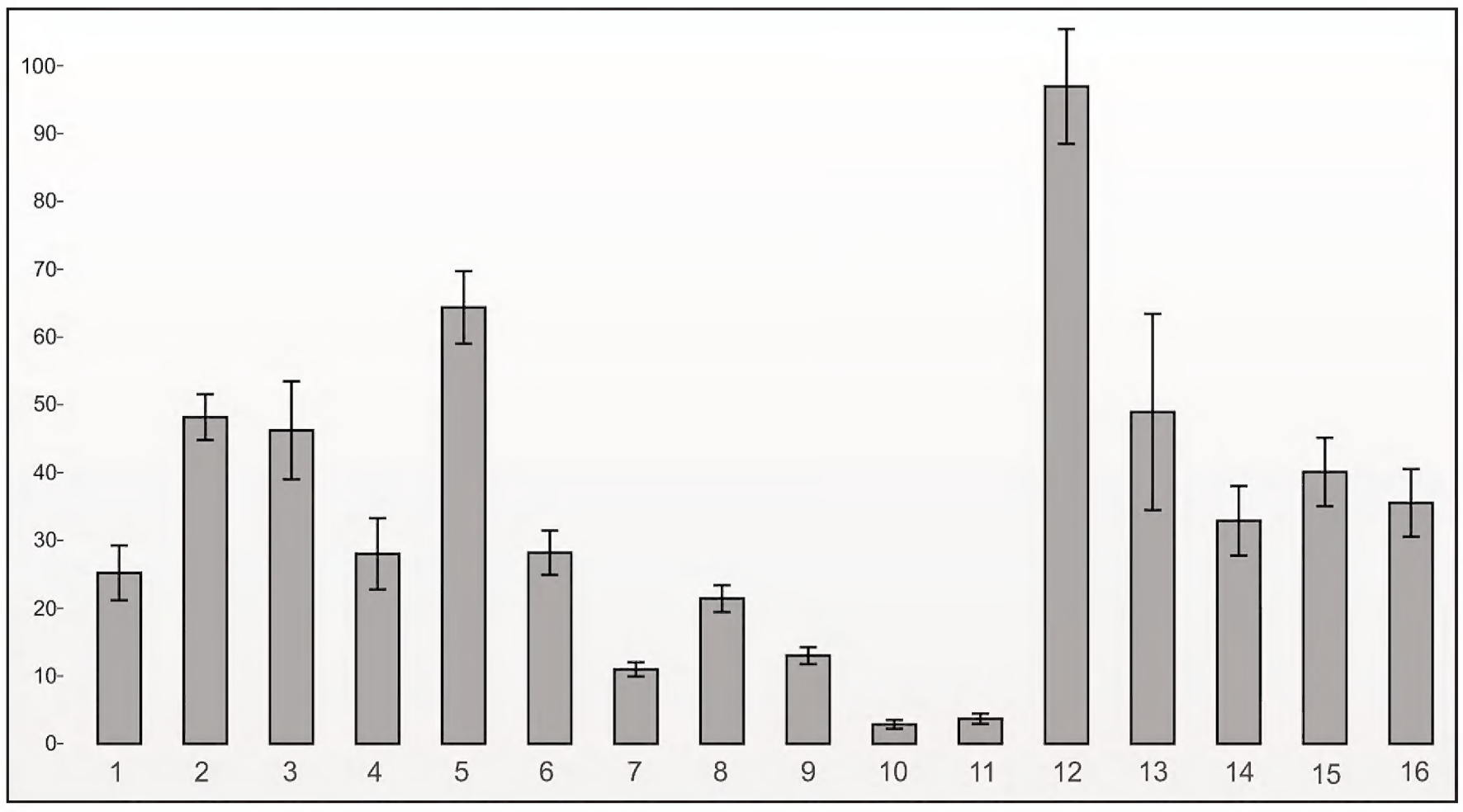

Figure 3. Graphic with mean and standard deviation of morphometric data from seven *Astyanax rivularis* populations. 1: predorsal distance; 2: prepelvic distance; 3: prepectoral distance; 4: preanal distance; 5: height of dorsal origin; 6: height of tail peduncle; 7: length of anal basis; 8: length of dorsal basis; 9: length of pelvic basis; 10: length of pectoral basis; 11: length of head; 12: head height; 13: snout length; 14: eye diameter; 15: interorbital distance; 16: jaw length.

1

Morphometrics	Range	Mean	SD
Standard length	41.44 – 80.07	63.90	12.19
	% of standard length		
predorsal distance	44.38 – 51.49	47.93	1.53
prepelvic distance	45.76 – 63.61	48.82	4.03
prepectoral distance	24.36 – 48.29	29.07	5.08
preanal distance	53.7 – 67.05	63.31	3.01
height of dorsal origin	23.21 – 33.07	26.37	2.50
height of tail peduncle	9.40 – 11.66	10.19	0.65
length of anal basis	19.36 – 26.69	22.36	2.25
length of dorsal basis	10.41 – 16.55	12.96	1.33
length of pelvic basis	2.34 – 4.28	2.89	0.49
length of pectoral basis	3.24 – 6.49	4.02	0.69
length of head	23.37 - 27.03	25.27	0.96
	% head length		
head height	78.97 – 108.94	91.8	6.53
snout length	49.88 – 68.65	58.46	4.56
eye diameter	28.30 – 39.44	32.89	3.52
interorbital distance	31.99 – 41.84	36.00	3.08
jaw length	28.33 – 49.21	37.04	5.04
Meristics	**Range**	**Mean**	**SD**
Scales in Lateral Line	33 - 39	35.63	1.83
Branched anal-fin rays	19 - 23	20.42	1.02

2

Morphometrics	Range	Mean	SD
Standard length	47.08 – 61.58	54.64	5.29
	% of standard length		
predorsal distance	46.65 – 53.22	49.94	2.31
prepelvic distance	43.92 – 48.53	45.04	1.82
prepectoral distance	25.31 – 28.63	28.29	1.40
preanal distance	61.52 – 69.30	65.41	2.69
height of dorsal origin	28.38 – 30.95	30.74	1.01
height of tail peduncle	11.04 – 13.13	11.89	0.84
length of anal basis	18.06 – 23.10	19.97	1.89
length of dorsal basis	11.87 – 14.19	13.62	0.98
length of pelvic basis	1.70 – 3.00	2.05	0.48
length of pectoral basis	3.20 – 4.17	3.87	0.42
length of head	19.55 - 22.60	20.72	1.16
	% head length		
head height	83.92 – 120.35	112.78	12.13
snout length	21.84 – 27.65	26.22	2.20
eye diameter	29.16 – 35.16	32.78	2.24
interorbital distance	32.55 – 50.73	44.14	6.02
jaw length	24.98 – 35.66	30.32	4.06
Meristics	**Range**	**Mean**	**SD**
Scales in Lateral Line	33 - 37	34.83	1.72
Branched anal-fin rays	17 - 20	18	1.10

3

Morphometrics	Range	Mean	SD
Standard length	61.68 - 82.73	70.36	6.84
	% of standard length		
predorsal distance	43.41 - 48.48	46.21	1.99
prepelvic distance	43.44 - 48.43	46.35	1.66
prepectoral distance	21.49 - 29.56	24.47	2.23
preanal distance	63.24 - 67.22	64.56	1.33
height of dorsal origin	22.06 - 28.95	25.16	1.83
height of tail peduncle	8.79 - 11.95	10.28	0.96
length of anal basis	17.67 - 22.58	19.84	1.39
length of dorsal basis	12.04 - 13.75	12.76	0.60
length of pelvic basis	1.85 - 3.86	3.00	0.62
length of pectoral basis	2.95 - 4.30	3.68	0.53
length of head	19.07 - 24.43	21.53	1.70
	% head length		
head height	86.31 - 100.00	93.87	4.79
snout length	42.55 - 58.38	50.55	5.23
eye diameter	22.52 - 35.11	27.63	4.18
interorbital distance	39.30 - 45.15	41.76	2.08
jaw length	33.42 - 40.62	36.21	2.28
Meristics	**Range**	**Mean**	**SD**
Scales in Lateral Line	33 - 39	36.4	1.84
Branched anal-fin rays	16 - 20	19	1.25

4

Morphometrics	Range	Mean	SD
Standard length	58.21 - 62.73	60.47	3.20
	% of standard length		
predorsal distance	54.69 - 57.58	56.14	2.04
prepelvic distance	47.51 - 50.59	49.05	2.18
prepectoral distance	28.33 - 29.46	28.90	0.80
preanal distance	61.87 - 68.65	65.26	4.79
height of dorsal origin	31.79 - 32.16	31.97	0.26
height of tail peduncle	12.53 - 13.06	12.79	0.37
length of anal basis	24.20 - 24.22	24.21	0.02
length of dorsal basis	12.73 - 15.16	13.94	1.72
length of pelvic basis	3.59 - 4.36	3.98	0.55
length of pectoral basis	2.87 - 4.93	3.90	1.45
length of head	28.67 - 28.73	28.70	0.04
	% head length		
head height	89.04 - 91.62	90.33	1.83
snout length	25.04 - 28.52	26.78	2.46
eye diameter	30.97 - 38.47	34.72	5.30
interorbital distance	33.57 - 37.39	35.48	2.70
jaw length	25.97 - 28.52	27.25	1.80
Meristics	**Range**	**Mean**	**SD**
Scales in Lateral Line	33 - 36	34.5	2.12
Branched anal-fin rays	22 - 22	22	0

Table 1. Morphometric and meristic data from Lage stream (n=19). Vouchers 1973, 1974, 1975, 1976, 1977, 1978, 1981, 1982, 1983, 1984, 1985, 1988, 1989, 1991, 1994, 2000, 2016, 2021, 2023. Table 2. Morphometric and meristic data from Açude Lote 94 (n=6). Vouchers 2937, 2938, 2939, 2940, 2941, 2944. Table 3. Morphometric and meristic data from Borrachudo stream (n=10). Vouchers 2048, 2050, 2051, 2052, 2053, 2054, 2069, 2070, 2072, 2073.Table 4. Morphometric and meristic data from Vereda Grande river (n=2). Voucher 2226, 2230.

Morphometrics	Range	Mean	SD
Standard length	34.95 - 56.72	44.94	9.25
	% of standard length		
predorsal distance	47.84 - 52.14	49.85	1.69
prepelvic distance	40.69 - 54.18	48.11	4.24
prepectoral distance	25.30 - 30.58	28.14	2.01
preanal distance	62.50 - 69.08	66.54	2.53
height of dorsal origin	25.19 - 43.89	31.28	5.97
height of tail peduncle	10.11 - 11.09	10.64	0.33
length of anal basis	19.92 - 22.65	21.03	1.03
length of dorsal basis	11.40 - 14.62	12.84	1.27
length of pelvic basis	1.98 - 2.79	2.35	0.26
length of pectoral basis	2.90 - 4.29	3.32	0.48
length of head	27.54 - 43.86	33.23	6.30
	% head length		
head height	83.07 - 105.22	93.46	9.43
snout length	53.91 - 93.42	62.60	13.79
eye diameter	25.71 - 54.39	34.38	9.22
interorbital distance	33.63 - 43.74	38.60	4.07
jaw length	31.29 - 40.16	36.23	3.34
Meristics	**Range**	**Mean**	**SD**
Scales in Lateral Line	30 - 38	34.14	2.67
5 Branched anal-fin rays	18 - 21	19.43	0.98

Morphometrics	Range	Mean	SD
Standard length	49.18 - 69.14	57.41	5.58
	% of standard length		
predorsal distance	29.82 - 51.47	46.48	6.19
prepelvic distance	21.43 - 52.88	36.16	14.18
prepectoral distance	14.59 - 45.80	32.15	10.04
preanal distance	61.74 - 67.61	64.39	2.18
height of dorsal origin	25.88 - 29.80	27.57	1.06
height of tail peduncle	9.83 - 13.91	11.23	1.08
length of anal basis	17.82 - 23.66	20.75	1.59
length of dorsal basis	11.57 - 18.18	13.57	1.90
length of pelvic basis	2.17 - 4.96	3.47	0.81
length of pectoral basis	1.79 - 5.53	3.32	1.33
length of head	19.58 - 27.11	23.25	2.40
	% head length		
head height	86.14 - 111.97	99.35	7.49
snout length	22.78 - 34.13	28.25	3.87
eye diameter	28.63 - 39.09	34.32	2.87
interorbital distance	34.46 - 52.10	43.15	7.00
jaw length	26.54 - 45.47	36.61	6.99
Meristics	**Range**	**Mean**	**SD**
Scales in Lateral Line	34 - 39	36	1.94
6 Branched anal-fin rays	17 - 23	19.5	1.27

Morphometrics	Range	Mean	SD
Standard length	56.6 - 73.16	61.15	4.22
	% of standard length		
predorsal distance	44.43 - 50.05	48.02	1.76
prepelvic distance	45.56 - 52.19	48.15	1.96
prepectoral distance	23.20 - 31.46	26.49	2.16
preanal distance	63.74 - 68.73	66.21	1.94
height of dorsal origin	28.83 - 32.39	30.32	1.11
height of tail peduncle	10.88 - 12.39	11.61	0.44
length of anal basis	18.74 - 24.09	22.13	1.38
length of dorsal basis	15.47 - 17.20	12.80	1.02
length of pelvic basis	1.99 - 3.36	2.56	0.46
length of pectoral basis	2.87 - 4.81	3.65	0.49
length of head	24.06 - 27.91	26.22	1.09
	% head length		
head height	98.03 - 114.11	104.42	4.80
snout length	51.47 - 60.39	55.49	2.77
eye diameter	28.75 - 51.12	34.69	5.35
interorbital distance	38.27 - 47.71	43.48	2.96
jaw length	32.34 - 46.03	35.71	3.34
Meristics	**Range**	**Mean**	**SD**
7 Scales in Lateral Line	32 - 38	35.2	2.04

Table 5. Morphometric and meristic data from das Éguas stream (n=7). Voucher 2169, 2170, 2172, 2172, 2173, 2175, 2177. Table 6. Morphometric and meristic data from Tiros stream (n=10). Voucher 2030, 2032, 2033, 2034, 2036, 2037, 2038, 2039, 2040, 2045. Table 7. Morphometric and meristic data from do Boi river (n=15). Voucher 2145, 2147, 2149, 2149, 2150, 2152, 2153, 2154, 2158, 2159, 2161, 2163, 2164, 2165, 2166.

DISCUSSION

Here we describe morphological diversity data from seven populations of *A. rivularis*.

Our data shows that in this region there is a huge diversity in size, shape and meristic counts in this species. Besides being considered a single valid species, separeted from *A. scabripinnis* according to the Catalog of Fishes (Eschmeyer & Fong, 2019), such diversity can indicate a high polymorphic species or we are dealing with a species complex.

Surveys in the Velhas River basin as a whole observed the occurrence of *A. scabripinnis* rather than *A. rivularis* (Alves & Pompeu, 2005) among the 107 species in the watershed, out of the 176

species of the São Francisco River in Minas Gerais (Alves et al., 1998, apud Alves & Pompeu, 2005). Meanwhile, a survey in the Serra do Cipó National Park identified three species of the genus *Astyanax* among the 36 species of fish collected in the Cipó River basin, a tributary of the Velhas River (Vieira et al., 2005). However, the presence of *A. rivularis* or Hasemannia nana was not reported, which led Triques (2006) to record the addition of these species to the list of fishes of the Serra do Cipó National Park. It was speculated that their absence in the first list was due to one of five factors, including that such species were collected but were not taxonomically recognized (Triques, 2006). Indeed, Vieira et al. (2005) reported the presence of *A. scabripinnis*, which suggests that the authors did not consider *A. rivularis* a valid species.

Casatti & Castro (1998) reports an occurrence of *A. rivularis* in the headwaters of São Francisco river. In their identification key for fishes of the São Francisco River basin, Britski and colleagues (1988) present it as *A. scabripinnis rivularis*, but do not record it in the list of the species found in the region of Três Marias - MG. Triques (2006) considers that, among other reasons, it is due to its absence in the region. However, there are reports from cytogenetic studies, where samples are identified as *A. scabripinnis*, in two populations close to the Três Marias (Minas Gerais State) region, with divergent diploid chromosome numbers – 2n=46 at Curral das Éguas populations and 2n=50 chromosomes at Viveiro de Mudas population (Moreira-Filho and Bertollo, 1991). Also in the region of Três Marias, Souza & Moreira-Filho (1995) identified 2n = 50 chromosomes for a population of *A. scabripinnis* from the Barreiro Grande Creek.

Lütken (2001) reports the morphological characteristics of *A. rivularis*, highlighting 33 to 38 lateral line scales; 5 to 6 rows of scales above the lateral line and 6 to 7 below it; on average, the number of ridged striations is high: 07-12-19 in larger specimens, 5 to 10 in smaller; 3 + 16 to 21 (mean 3+18) rays in the anal fin; head length contained 4 times or slightly less in standard length; height contained about 3 times in the standard length; eye diameter of 3 and 1/3 to more than four times in the length of the head; The infra-orbital bones are usually somewhat arched and well-equipped with furrowed grooves; the jawbone usually reaches up to quite below the eyes, more rarely just up the vertical line drawn from its anterior edge; there are 5 intermaxillary teeth in first series and jawbone with 1 to 3 small teeth; the dorsal fin height is generally smaller than its distance from the adipose fin; the tip of the pectoral fin never reaches the ventral fin. Lütken further comments that *A. rivularis* closely resembles *A. fasciatus* (treated by him as *Tetragonopterus cuvieri*) both in body shape and in general appearance.

Therefore, an effort from different areas of science is necessary to better characterize the natural history and geographical distribution of this species in the São Francisco basin, considering that, through DNA "barcoding" technique, Carvalho and colleagues (2011) observed only 0.93% divergence between *A. rivularis* and *A. fasciatus* Cuvier, 1819, another species that has been very difficult to classify, since it was first described in the rivers of Brazil. Besides, different chromosome numbers in the different populations studied suggest that even *A. rivularis* in São Francisco River (data not shown) may represent more than one Operational Taxonomic Unit, thus highlighting the need for further studies in the region.

ACKNOWLEDGEMENTS

The authors are thankful to William Lopes Silva, Rafael Henrique Fernandes, Denis Glauber da Rocha Reis, Rodrigo Desordi, and Pierre Rafael Penteado (Rio Paranaiba, MG, Brazil) for the collection of samples. This work was developed with funding provided by the Foundation for Research Support of the State of Minas Gerais (Fundação de Amparo à Pesquisa do Estado de Minas Gerais, FAPEMIG) and the Universidade Federal de Viçosa, Rio Paranaíba Campus.

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Biodiversity Journal, 2019, 10 (4): 315–320 https://doi.org/10.31396/Biodiv.Jour.2019.10.4.315.320

Coexistence of *Danaus chrysippus* (Linnaeus, 1758) (Lepidoptera Nymphalidae) on the Milkweed *Pergularia tomentosa* L. (Asclepiadaceae) in Aïn Naga (Biskra, Algeria)

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ABSTRACT A butterfly species, *Danaus chrysippus* (Linnaeus, 1758) (Lepidoptera Nymphalidae), also known as African Queen, is recorded for the first time in the arid region of Aïn Naga (Biskra, Algeria). Adults of *D. chrysippus* were recorded in a survey from October 2018 to February 2019 on their host plant *Pergularia tomentosa* (Milkweed) (Asclepiadaceae). Additional data on the life cycle and behavior of this species are given.

KEY WORDS Butterfly; biodiversity; bioconservation; Danainae; distribution.

Received 24.06.2019; accepted 25.10.2019; published online 31.10.2019

INTRODUCTION

Danaus chrysippus (Linnaeus, 1758), also known as African Queen, is a butterfly belonging to the family Nymphalidae that includes about 7000 species worldwide. It is a migrant butterfly belonging to Danainae, a subfamily of Nymphalidae - tropical butterflies with a complex biology (Francke, 1989).

Danaus chrysippus is widespread in Africa, southern Europe (Canary Islands, coastal Mediterranean regions, Turkey), Saudi Arabia, tropical Asia, Australia and New Zealand.

Danaus chrysippus has considerably increased and extended its range in the North African coastal regions, probably due to warmer climates, and from there has colonised parts of the south coast of Spain, Corsica, Sardinia, Sicily, Italy, Malta, and Greece (Burton, 2001).

This species is multivoltine and lives in open areas, sometimes even degraded, and near gardens or cultivated areas (Perković, 2006). The biology of this species is influenced by the availability of larval foodplants. Altough it is polyphagous, its larvae feed on plants which contain cardenolides, expecially Asclepiadaceae, Apocynaceae and Moraceae (Ackery & Vane-Wright, 1984).

There is a huge literature surrounding this species. Particularly, taxonomic studies are reported by Seitz' (1927) and Munroe (1961), Downey (1962), Pierre (1984). Ackery & Vane-Wright (1984) use the host plant relations as data for butterflies classification. Igarashi (1984) classified the Papilionidae mainly based on the morphology of their immature stages.

However, there is no reason to suppose that host plant associations can be used for taxonomy with respect to morphological characters (Ackery, 1988).

In this work, based on photographs of the study area during the period October 2018–February 2019, we expose and confirm the coexistence of *D.*

chrysippus on the Milkweed *Pergularia tomentosa* L. (Asclepiadaceae) in the arid region of Aïn Naga (Biskra, Algeria).

MATERIAL AND METHODS

In our research in Aïn Naga (Biskra, Algeria) (Fig. 1) during a period from October 2018 and February 2019, our purpose was to realize a survey on the Milkweed *Pergularia tomentosa* (population density, height, leaves number, pods and seeds number, etc…) and collect some seeds to test its seed germination under drought conditions under greenhouse.

Our study area is about 320 m x 170 m so about 5 ha with > 50 individuals of *P. tomentosa* per 1000 m^2. An average of 20 °C of temperature and 25% of humidity was recorded during this period between 2h00 and 4h00 pm.

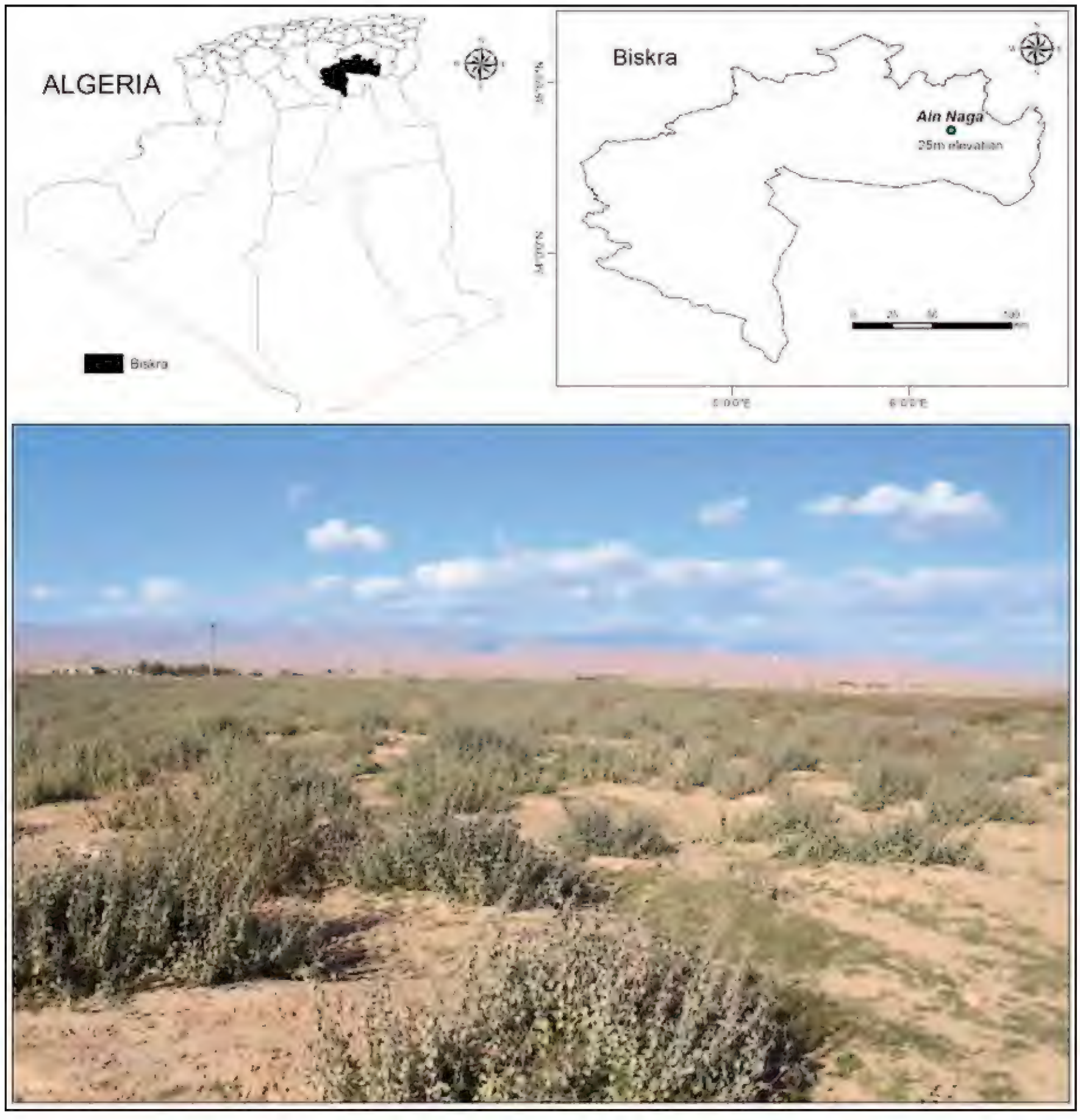

Figure 1. Study area where *Danaus chrysippus* was observed (Aïn Naga, Biskra, Algeria).

During this survey, several specimens of *D. chrysippus* were observed especially on this plant species. Thus, we decided to take pictures with a Coolpix Nikon P610 and send them to the MLMP (The Monarch Larva Monitoring Project, University of Minnesota) for identification, confirming that the butterfly is *D. chrysippus*.

RESULTS

During the study period (October 2018-February 2019) we studied the *Danaus* Kluk, 1802 populations found in the arid region of Aïn Naga (Biskra, Algeria) in relation to the presence of *P. tomentosa*.

Every week we recorded about 18-23 specimens of this butterfly (both sexes) and we found a coexistence between *D. chrysippus* and its host plant *P. tomentosa*. Coexistence was justified by the stages of butterfly development (larval, pupal and adult stages) on this milkweed (Fig. 2).

DISCUSSION

Biological cycle of the butterflies of the subfamily Danainae is linked mainly to Asclepiadaceae, Apocinaceae and Moraceae but also to Convolvulaceae, Euphorbiaceae, Sapindaceae, and Plumbaginaceae (Ackery & Vane-Wright, 1984). Particularly, the larvae of *D. chrysippus* feed mainly on Asclepiadaceae, from which they store cardenolides (MEBS et al., 2005) to escape predators.

Danainae have a great diversity among almost all factors relating to ecology of the host plants with interesting correlations (Brower et al., 2010).

Currently, there has been no up-to-date review of the chemoecology of Danaini since Ackery & Vane-Wright (1984). This work has glossed also over a number of supposed exceptions and problems due to under-sampling, insufficient chemical analyses, and unreliable identifications; moreover, many species were not included in this study because of the difficulty of obtaining living specimens.

Another very important plant relationship for most Danaini, including *D. chrysippus*, is adult pharmacophagy, notably, the adult uptake of pyrrolizidine alkaloids from various plants that are not larval hosts. Ackery & Vane-Wright (1984) have records of adult danaines attracted to over thirty families of flowering plants, bur many of these are certainly only nectar sources. The main host plants of *Danaus* are numerous asclepiads, a group now placed within the Apocynaceae. The utilization of *Panowla* (Apocynaceae) both as a larval foodplant and adult attractant has led to the suggestion that *Parsonsia* might represent the ancestral foodplant of the Danainae (Edgar et al., 1974; Edgar, 1984).

With respect to *D. chrysippus* in Algeria, Tennent (1996) notes that it has been "*recorded commonly on* Pergularia tomentosa *and* Cynanchum acutum", and, probably, also on *Calotropis procera*. According to Samraoui (1996), the breeding status of *D. chrysippus* in the Algerian Sahara is confirmed and its larval foodplant on *Calotropis procera* is reported on. Moreover, the species has apparently increased in numbers and considerably expanded its range. However, in the last two decades, no study has been able to determine a specific range and distribution of this species in Algeria. There are several inventories in some arid and Saharan region of Algeria that include observations of very few individuals.

Borgo et al. (1992) reported that the distribution of *D. chrysippus* in Italy from 1986–1990 was linked to the warm conditions.

The climatic data in the region of Biskra during the period 1980-2018 taken from the universal climate data site "Tutiempo" (www.tutiempo.net) shows that the average annual temperature is 22.7 °C, the maximum annual average is 28.4 °C, and the minimum annual average is 16.9 °C. The total annual precipitation is 160.6 mm with only 30 days of rainy days.

CONCLUSIONS

In conclusion, these brief observations on the presence of *Danaus* in Algeria and its trophic relations with *Pergularia* constitute a further contribution to the biology of this interesting butterfly.

We need more data and information on this butterfly in Algeria (distribution and density) to consider it a vulnerable or endangered species, but it is important to point out these observations to try to protect its habitat in this new study area.

Figures 2–7. *Danaus chrysippus* and *Pergularia tomentosa* from Aïn Naga (Algeria). Fig. 2. Adult butterfly feeding-nectar flower. Fig. 3. Butterflies mating. Fig. 4. Butterfly eggs hatching. Figs. 5-7. Leaf Feeding Caterpillars (larvae).

Figures 8–13. *Danaus chrysippus* and *Pergularia tomentosa* from Aïn Naga (Algeria). Figs. 8, 9. Caterpillars. Fig. 10. Larvae preparing for pupa stage. Fig. 11. Pupa. Fig. 12. Butterfly emerging from a chrysalis. Fig. 13. Adult butterfly.

ACKNOWLEDGENENTS

The authors are very grateful to Dick Vane-Wright (Affiliate Member, Durrell Institute of Conservation and Ecology (DICE), University of Kent, Canterbury CT2 7NR), Katie-Lyn Bunney (Program Coordinator - Monarchs in the Classroom, The University of Minnesota Monarch Lab) and Karen Oberhauser (Director, University of Wisconsin-Madison Arboretum, The University of Minnesota Monarch Lab) for their help in confirming the butterfly identification and taxonomy. We thank also Arvind Singh (PhD and Researcher at the Department of Botany, Banaras Hindu University Varanasi, India) for helping us confirming the identification of *Pergularia tomentosa*.

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Biodiversity Journal, 2019, 10 (4): 321–324 https://doi.org/10.31396/Biodiv.Jour.2019.10.4.321.324

First detection of the "flowerpot snake" *Indotyphlops braminus* (Daudin, 1803) (Serpentes Typhlopidae) in Ischia (Italy): a new possible invasive species

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ABSTRACT

Indotyphlops braminus (Daudin, 1803) (Serpentes Typhlopidae), also known as "flowerpot snake", is a small subterranean blind snake, which holds the widest distribution on the globe. This species, by taking usually refuge in pot plants, has been indirectly transported by humans via the main trade routes. Close to Europe, *I. braminus* has been reported in Macronesia (Canary Islands and Madeira) and Northern Africa; while in Europe it has been detected in the Balearic Islands (specifically in Mallorca) and one population was also found in the Province of Almería, in the southern Iberian peninsula. Up to date, no further reports were made in Europe. With this note, we report the first Italian observation of *I. braminus*, specifically in Ischia Island. As for other alien species, an early detection of allochthonous populations plays a pivotal role to activate specific and useful management strategies.

KEY WORDS Allochthonous; flowerpot snake; *Indotyphlops braminus*; Ischia; Italy.

Received 20.10.2019; accepted 03.11.2019; published online 19.11.2019

INTRODUCTION

Introduction and spread of alien species are considered one of the main threats to biodiversity at different scales. Indeed, this is mainly due to the rates at which specific species are introduced, as well as their possible destructive impact on native biota (Rato et al., 2015). In this spectrum, reptiles are usually involved both as alien invaders and as native sufferers (Rato et al., 2015). Accordingly, an early detection of allochthonous populations plays a pivotal role to activate specific and useful management strategies (Rato et al., 2015).

Indotyphlops braminus (Daudin, 1803) (Serpentes Typhlopidae) is a small subterranean blind snake, originating from Indo-Malayan region, showing a length ranging between 120 mm and 230 mm (Bamford & Prendergast, 2017). Twenty rows of scales around the body and 292–368 transverse rows characterize the body scales in this species (Figs. 1, 2). In order to burrow in the soil, the rostral plate and the two nasal plates protrude from the mouth to form a sort of rounded shovel (Geniez, 2018). The nasal plate is divided by a furrow, which is directly in contact with the pre-ocular scale (Geniez, 2018) while the head shows typical rows of whitish cephalic glands (Fig. 3) (Wallach, 2009). An obtuse, conical spine characterizes the terminal caudal vertebra, distinguishing the tail from the rest

of the snake's body (Fig. 4) (Bamford & Prendergast, 2017).

Indotyphlops braminus is mainly fossorial and rarely comes to the surface. When it rains and the soil becomes humid and waterlogged, the snake finds it easier to move across the surface to find food or new sites to colonize (Bamford & Prendergast, 2017). The diet is mainly characterized by ants and termites (including larvae and pupae), beetle larvae, small flies, fungi and even insect excrement (Bamford & Prendergast, 2017).

As reported above this snake is restricted to warm areas with high humidity, usually in the tropics. However, *I. braminus*, among the snakes, holds the widest distribution on the globe (Rato et al., 2015; Bamford & Prendergast, 2017). Indeed, this species, by taking refuge in pot plants (from which it takes the name of "flowerpot snake"), is indirectly transported by humans via the main trade routes (Bamford & Prendergast, 2017). Furthermore, its parthenogenic reproduction increases its worldwide distribution, allowing the colonization of several sites in Asia, Oceania, Africa and Americas (Rato et al., 2015; Kamosawa & Ota, 1996).

Close to Europe, *I. braminus* has been reported in Macronesia (Canary Islands and Madeira) and Northern Africa, while in Europe it has been detected in the Balearic Islands (specifically in Mallorca) and one population was also found was found in the Province of Almería, in the southern Iberian peninsula (Zamora, 2017). To our knowledge, to date, no further reports were made in Europe.

MATERIAL AND METHODS

Ischia is a volcanic and mountainous island in the Tyrrhenian Sea that lies at the northern end of the Gulf of Naples, about 30 kilometers from the city of Naples. As in the main Spanish sites (Balearic Islands and Southern Iberic peninsula), Ischia's climate is Mediterranean with dry summers and mild wet winters, further justifying the survival of this snake in the little Italian island.

The site where our observations were made is a private suburban garden at 40°44.3130' N 13°56.9060' E, 67.3 meters above sea level and about 100 meters from the sea. The site is characterized by lava soil, gardening plants and grass, while the surrounding area by Mediterranean scrub and pine trees (*Pinus pinaster* Aiton and *Pinus halepensis* Miller).

RESULTS

Systematics

Classis REPTILIA Laurenti, 1768
Ordo SQUAMATA Oppel, 1811
Subordo SERPENTES Linnaeus, 1758
Familia TYPHLOPIDAE Merrem, 1820
Genus *Indotyphlops* Hedges, Marion, Lipp, Marin et Vidal, 2014

Indotyphlops braminus (Daudin, 1803)

The first observation in this area dates back to 24 months ago, with a mean observation of 15 specimens per month and an increase of observations during the rainy days and summer season (June-September). According to what reported above, if it is true that that the dispersal capacity of this snake is limited (because of its reduced mobility and underground customs, which require loose soils for gallery burrowing), at the same time, its adaptability to lava soil remains interesting in Ischia island as well as in Macronesian islands.

Finally, as in the main Spanish populations (including Canary Islands), the proximity of the site of our observation to the sea, stress the fact that the high humidity and temperature stability create an ideal habitat for the survival and reproduction of this species (Zamora, 2017).

DISCUSSION AND CONCLUSIONS

Concluding, as far as we know, this is the highest latitude in which a population of this species has been found and this is the first observation of *I. braminus* in Italy. Based on its small size, on its elusiveness, fossil ecology and diet, the ecological and economic impact of this species in the native biota is not yet known.

At the same time, the evaluation of a possible impact on the native arthropod communities, with a relative involvement in the entire trophic chain,

Figures 1–4. *Indotyphlops braminus*. Fig. 1: the eyes are slightly perceptible as small dots under the head scales. The coloration varies from light brown to black dorsally, while it's usually lighter ventrally. Fig. 2: *I. braminus* is a small snake, showing a total length ranging between 120 mm and 230 mm. Here, the comparison with a coin of 5 cents. Fig. 3: the head shows rows of the typical whitish cephalic glands. The glands have been described as forming "a faint crenellated whitish marginal line" (Wallach, 2009). Figure 1d. The tail is very short and is characterized by a conical spine capping the terminal caudal vertebra.

must be carefully evaluated. Besides, considering that our observation involved a small island, where geographical isolation and the scarce dispersion can further influence the native species, the study of a possible future impact of *I. braminus* deserves deeper investigation. A careful control above all of gardening and careful instructions to gardeners and florists, can play a pivotal role in the primary prevention, avoiding and/or reducing new introductions.

ACKNOWLEDGENENTS

We thank you Mr. Simone De Santis (Ischia, Italy) for the photographic contribution and Mrs. Rosanna Magno (Ischia, Italy) for its courtesy and helpfulness.

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Biodiversity Journal, 2019, 10 (4): 325–328 https://doi.org/10.31396/Biodiv.Jour.2019.10.4.325.328

Morphological and genetic confirmation of extensive distribution of a pelagic polychaete *Poeobius meseres* Heath, 1930 (Annelida Flabelligeridae)

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ABSTRACT A pelagic polychaete, *Poeobius meseres* Heath, 1930 (Anellida Flabelligeridae), is mainly known from California, Northeast Pacific, and is well studied in the region. However, little is known from Northwest Pacific, which is needed for understanding its biodiversity and accurate distribution. We report morphological and genetic data of this species collected off Tohoku, Japan and off the Aleutian Islands, USA, and confirm ITS extensive distribution (over 4000 km).

KEY WORDS Aleutian Islands; pelagic; Polychaeta; polychaetes.

Received 21.04.2019; accepted 19.06.2019; published online 11.12.2019

INTRODUCTION

Pelagic polychaetes often have wide-range distribution due to their pelagic habits (Dales & Peter, 1972). However, their distributional pattern is not well clarified due to the difficulty of sampling across several countries for covering broad distribution. Further information on their distribution is needed for understanding their life cycle and biogeography.

A pelagic polychaete genus, *Poeobius* Heath, 1930, is unique in its morphology, i.e., it lacks segmentation/chaetae and was classified as Poeobiidae that was thought to be the key for understanding the connectivity between polychaetes and echiurans (Heath, 1930). Recent molecular phylogenetic analyses showed that this genus is contained in Flabelligeridae (Burnette et al., 2005; Osborn & Rouse 2008, 2010). *Poeobius meseres* Heath, 1930 is the sole species of *Poeobius* and is distributed in the Pacific Ocean (Heath, 1930; McGown, 1960; Salazar-Vallejo, 2008). Recently, an undescribed species of *Poeobius* was found in the Atlantic Ocean (Christiansen et al., 2018). *Poeobius meseres* was reported mainly from Northeast Pacific (Salazar-Vallejo, 2008). However, some of the papers indicated that the species is also present in Northwest Pacific and Southeast Pacific (Yamada, 1954; McGown, 1960). Salazar-Vallejo (2008) reviewed specimens of *P. meseres* collected from Northeast Pacific and concluded there are at least two morphotypes in this species. Salazar-Vallejo (2008) also mentioned "some other records in the Western Pacific and in subtropical localities should be confirmed" for a better understanding of *Poeobius* diversity. Although there are records by McGown (1960) of *P. meseres* from Japanese waters, the paper did not provide any morphological and genetic data, and thus, we could

not infer that the distribution record consists of one or more species in Pan-North Pacific. Morphological information accompanied with its genetic data are needed for understanding of the biodiversity and distribution of *Poeobius* species. In this study, we report *P. meseres* collected from Aleutian and Japanese waters with morphological and genetic confirmation.

MATERIAL AND METHODS

Sampling from two localities: i) Vertical Multiple Plankton Sampler (VMPS) was applied on 6 August 2017, three specimens were collected from 750–1000 meters of depth, off Aleutian Islands (54°17.7229' N, 166°23.7795' W), USA, during the MR17-04 cruise by R/V Mirai; ii) ROV Hyper-Dolphin was operated on 9 October 2013, one specimen was collected from 433 meters of depth, off Sanriku (39°36.984' N, 142°15.543' E), Japan, during the NT 13-21 cruise by R/V Natsushima. All the specimens were fixed and preserved in 70% ethanol. The preserved specimens were observed under stereoscopic microscopes (Nikon SMZ1500 and OLYMPUS BX51); photographs were taken using a digital camera (Nikon D5200). Voucher specimens were deposited in the JAMSTEC (No. JAMSTEC-1170056154 and 1130040972). DNA extraction, sequencing, and phylogenetic analysis were conducted by use of cytochrome c oxidase subunit I (COI) following the method of Jimi & Fujiwara (2016). The genetic distance was calculated following Jimi & Fujiwara (2016). Newly obtained sequences have been deposited in the DNA Data Bank of Japan (DDBJ): DDBJ No. LC508300 (Aleutian), No. LC508299 (Japan). Additional COI sequences of *Poeobius meseres* from California and *Trophoniella hephaistos* from Japan as an outgroup were obtained from GenBank (GenBank No. EU694130 and LC136932) (Osborn & Rouse 2008; Jimi & Fujiwara 2016). COI gene of *Daylithos* sp., another member of the outgroup, was sequenced and deposited in DDBJ (DDBJ No. LC508301.

RESULTS

Systematics

Phylum ANNELIDA Lamarck, 1809
Classis POLYCHAETA Grube, 1850
Ordo TEREBELLIDA sensu Rouse et Fauchald, 1997
Familia FLABELLIGERIDAE Saint-Joseph, 1894
Genus *Poeobius* Heath, 1930

Poeobius meseres Heath, 1930

EXAMINED MATERIAL. JAMSTEC 1170056154, three specimens, off Aleutian Islands, USA, collected by Naoto Jimi and Atsushi Yamaguchi, 6 August 2017. JAMSTEC 1130040972, one specimen, off Sanriku, Japan, collected by Yoshihiro Fujiwara, 9 October 2013.

DESCRIPTION. Body cylindrical, tapered in anterior and posterior region, cuticle thick, transparent in life and after preservation (Fig. 1). Body surface without papillae and sands. Eyes absent. Palps same length with branchiae or slightly shorter. Branchiae cirriform, arranged in a continuous dorsal series, ten in number. Single nephridial lobe present, placed close to the branchial filaments. Cephalic cage absent. Parapodia absent. Chaetae absent. Black eggs contained in the body. Pelagic.

MOLECULAR ANALISYSES. In the resulting tree, specimens from the Japan and Aleutian Islands formed a clade (Bootstrap [BS] value: 100 %) (Fig. 2). They differed 0.02 in terms of the K2P distance. The specimen from the California is sister to Japan/Aleutian clade, with high BS value (100 %). They differed 0.055–0.057 in terms of the K2P distances.

Figure 1. A live specimen of *Poeobius meseres* from off the Aleutian Islands.

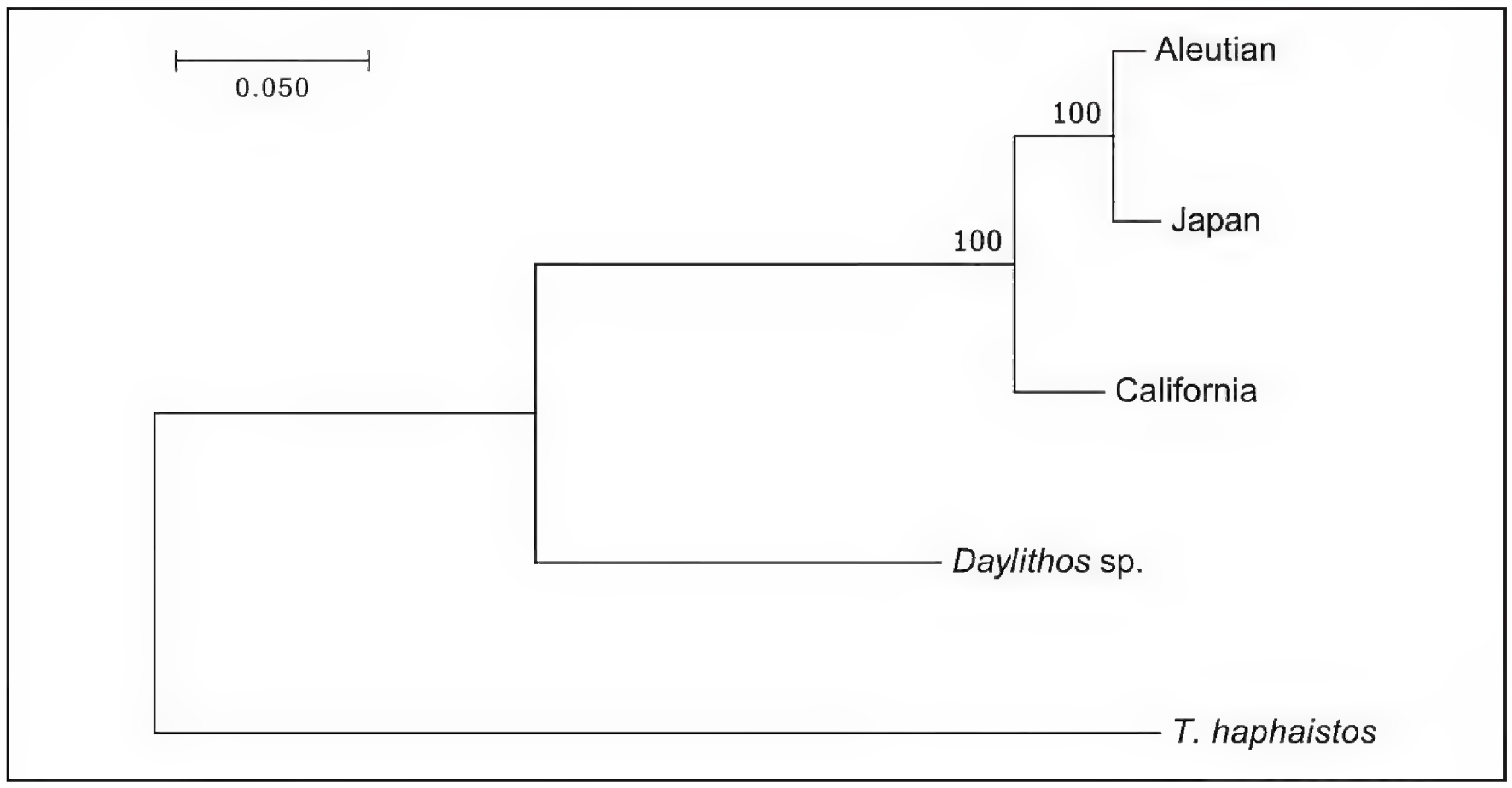

Figure 2. ML tree of *Poeobius messers* from off Japan, off the Aleutian Islands, off California based on COI gene sequences. *Trophoniella hephaistos* and *Daylithos* sp. are used as an outgroup. Numbers indicate bootstrap nodal support values.

DISCUSSION

In morphological observation (Fig. 1), the *P. meseres* specimens have the following features as reported in previous studies (Salazar-Vallejo, 2008): i) body depressed without segmentation, parapodia or chaetae, ii) transparent cuticle, iii), the presence of branchiae and nephridial lobe, iv) pelagic. Other features completely align with the previous genus diagnosis. In genetic analysis, Aleutian and Japanese specimens form a monophyletic clade (Fig. 2). The genetic K2P distance between Aleutian-Japan was 0.02. On the bases of the morphological identity between Japanese and Aleutian individuals, this genetic distance seems to be intraspecific variations. On the other hand, between Aleutian/Japan-California there were 0.055–0.057. There is no conclusive proof that this genetic distance is intraspecific variation because detailed morphology of the Californian specimen used for the molecular analysis was not shown. According to the description of Salazar-Vallejo (2008), *Poeobius meseres* contained at least two morphological variations. Our specimens are identical with one of the morphological variations in the following features: i) single nephridial lobe; ii) 10 branchiae; iii) branchiae are the same length or slightly longer than palps. However, there is no information on the features from the Californian individuals used for the molecular analysis. Genetic connectivity between individuals collected from Japanese and Californian waters is not discussed due to the lack of genetic information. More sequences with morphological data are needed for understanding of the Pan-North Pacific connectivity of this species.

Recently, studies about their "cosmopolitan" nature revealed the limited distribution of the polychaete species in a certain area (Hatchings & Kupriyanova, 2018). Pelagic polychaetes have been thought to be cosmopolitan based on morphological analysis, but the genetic confirmation is critical for understanding their accurate distribution. Our morphological and genetic data support extensive distribution (about 4000 km) of the pelagic species.

ACKNOWLEDGENENTS

We thank the crews of the R/V Mirai and Natsushima, scientific members of the cruise MR 17-

04 and NT 13-21 for them help in the sampling. This study is partly supported by JSPS KAKENHI No. JP17J05066 to NJ.

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Biodiversity Journal, 2019, 10 (4): 329–336 https://doi.org/10.31396/Biodiv.Jour.2019.10.4.329.336

Melissopalinological study of Sicilian honey by morphological and molecoular approach

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ABSTRACT With the Legislative Decree n.179 of 21 May 2004 on the unifloral honey's characteristics and with the CEE regulation 2081/92 of DOP denomination (from protect origin), scientific investigations in the agri-food sector started to occupy a prominent position with increasing importance. This area of investigation has been considerably deepened, becoming fundamental in order to consider this term valid, as it is also fundamental for investigation on production chain and denomination, made on various types of products. The morpho-genetic characterization analysis of pollen from honeys, made in the present work, lands itself well to this purpose; with the aim of ascertaining the validity of the wording of "unifloral" applied to some products from the beekeeping industry. Since the implications to which the investigations in the agri-food sector lead to cover not only the purely scientific-agronomic, but also the legal field, it follows the importance of an increasingly scientifically accurate methodology that also allows a faster processing of the sample. The protocol commonly used for pollen characterization of honeys is based on the visual recognition of the pollen present in the sample, their count and statistical analysis of the data obtained. Our method, using Real Time PCR technology, allows a qualitative and quantitative analysis of the pollen species inherent in the sample, thus allowing a fast and accurate analysis of the data that lends itself well to assist the classical research based on visual recognition of pollen.

KEY WORDS Honey; pollen; agri-food sector; DOP; PCR.

Received 11.07.2019; accepted 21.10.2019; published online 11.12.2019

INTRODUCTION

The Legislative Decree n. 179 of 21 May 2004 cites the characteristics of unifloral honey. These must have precise chemical-physical parameters (content of sugars, water, insoluble substances).

It also sets the minimum purity values to consider unifloral honey. In Italy, the Community Directive 74/409 of 22/7/74, which regulates the marketing of honey, defines the general criteria of composition and also provides for the possibility of declaring the botanical origin of honey, without offering the necessary means to identify the unifloral honeys.

The aim of the study is to be able to perform an evaluation, both through microscopic analysis and through biomolecular techniques, of the quantity and type of pollen granules present in the various commercialized unifloral honeys, in order to detect any case of fraud.

For this reason we have tried to optimize a multidisciplinary survey system for honey certification.

This type of investigation initially envisaged a phase of microscopic observation, aimed at the

study and recognition of the pollen morphologies among the various floral species through the study of the exine, the outermost wall of the pollen grains.

Generally mature pollen grains have variable dimensions and are covered by a layer of lipids and carotenoids which facilitate their adhesion on the stigma surface. The pollen has a protective layer composed of two walls: intine and exine.

The intine is constituted by polysaccharides such as cellulose and hemicellulose, the exine is constituted by Sporopollenin.

The presence of structures and ornamentations on the outermost portion of the exine allows to attribute a certain pollen morphology to a well-defined botanical species.

MATERIAL AND METHODS

The present study was carried out in the palynological and melissopalinological field, through the analysis of pollen granules of Mediterranean floral species and predominantly unifloral honeys (Pignatti, 1982; Accorti et al., 1986; Persano Oddo et al., 1991, 1995; Serra Bonvehí & Granados, 1993; Franck et al., 2000; Jerkovic et al., 2008; Lolli et al., 2008; Attenzio et al., 2016; Rodopoulou et al., 2017; Tariba et al., 2018; Can et al., 2015; Mannina et al., 2015; Marengo et al., 2017).

The palynological recognition consists in the analysis of the pollens that are extracted by washing the anthers through the use of ethyl ether, $(C_2H_5)_2O$.

The pollens that have been analyzed through this technique belong to some of the main botanical families used for the production of honey (*Citrus* spp., *Eucalyptus* sp., *Rosmarinus officinalis*, *Acacia* sp., *Heliantus annuus*).

Initially, microscopic observations were made without the use of coloring agents to analyze the pollen grains in their natural color which may change according to the botanical family.

In order to make the palynological recognition more efficient, we also created a colored preparation with fuchsin.

Fuchsin (0.1% alcohol solution) is prepared adding 100 mL of Ethanol (Et-OH) per 0.1g of basic fuchsin.

The color with the fuchsin makes more evident the morphological characteristics of the pollen grain, exine in particular, by observation under an optical microscope.

Observation without fuchsin

We proceed with the collection, in the field, of flowers and inflorescences with anthers loaded with pollen grains which present an entomophilous pollination.

After that, we proceeded to remove the anthers from the rest of the flower. Once removed, we wash with ethyl ether (1–2 ml) inside a porcelain capsule, to allow the release of the pollen grains, which will settle on the bottom.

At the end, let the ethyl ether, contained in the porcelain capsule, be decanted under a chemical hood. As soon as the ethyl ether has evaporated completely, we can see that the pollen grains have settled on the bottom of the capsule.

If there are some floral parts in the porcelain capsule, detached after washing with ethyl ether, it is necessary to remove them.

Subsequently, 150 µl of distilled H_2O will be taken with a micro pipette and poured into the porcelain capsule containing the pollen, making "up and down" with the same pipette in order to bring as many pollen grains as possible into solution. Now, 50 µl of distilled H_2O containing the pollen will be taken and placed on a glovebox.

We will wait for the evaporation of the distilled H_2O (at room temperature or at a temperature not higher than 40 °C) to proceed to the observation of the pollen grains with an optical microscope (Figs. 1–4).

Observation with fuchsin

First of all the glycerine gelatin is liquefied through a thermostatic bath or in a microwave oven for no more than 5 seconds.

Since the pollens have a different affinity towards fuchsin, it is advisable to prepare a set of glycerine gelatin containing the basic fuchsin in different concentrations (from 0.2 to 1.5 ml of fuchsin every 10 ml of glycerine gelatin).

Once the basic fuchsin has been added, we stir it to obtain a homogeneous mixture.

For the creation of the colored preparation the same steps were carried out for the non-colored preparation, adding glycerine gelatin containing

Figures 1–4. Pollen granules without fuchsin: *Asphodelus* sp. (Fig. 1), *Carduus galactites* (Fig. 2), *Rosmarinus officinalis* (Fig. 3), *Prunus dulcis* (Fig. 4). Figures 5, 6. Pollen granules with fuchsin: *Prunus dulcis* (Fig. 5), *Rosmarinus officinalis* (Fig. 6), *Ailanthus altissima* (Fig. 7), *Citrus aurantium* (Fig. 8).

fuchsin before observation under a microscope.

15 µl of gelatine containing the fuchsin are taken and poured onto the glove containing the pollen.

At this point a coverslip will be applied to distribute uniformly the gelatin containing fuchsin.

Once the gelatine containing the fuchsin has solidified we can proceed to the observation under an optical microscope (Figs. 5–8).

All the steps were made working with a chemical hood, due to the presence of volatile toxic substances.

Melissopalinological recognition

After the recognition of the pollen morphology of the harvested floral species, through the optical microscope, it was possible to proceed with the melissopalinological analysis through the extraction of the pollen grains from various commercialized honey and to the microscopic observation, in order to perform an initial analysis compositional (Loveaux et al., 1978).

We take 10 g of honey and place it inside a 50 ml Falcon. Subsequently, 40 ml of distilled water are poured into the same Falcon, stirred to mix and place the Falcon in a thermostatic bath for about 30–40 minutes (depending on the honey density) in order to completely melt the honey in water.

After this step, the Falcon containing our sample will be centrifuged at 15,000 rpm for 15 minutes to separate the solid phase, containing the pollen grains, from the sugars and from the waxes that are present in honey.

After centrifugation the liquid phase is removed and it is possible to observe the sediment, consisting of the pollen grains, on the bottom of our Falcon.

To make sure that most of the sugars and waxes are effectively removed, which could interfere with both microscopic observation and DNA extraction, our sediment is removed, transferred to a 15 ml Falcon and suspended again in 10 ml of distilled water. Then, the steps of the thermostatic bath and centrifugation at 15,000 rmp for 15 minutes will be repeated.

Now it is possible to remove the liquid phase again. The pollen grains on the bottom are suspended in 100 µl of distilled H_2O and placed on a glove slide and viewed through a microscope.

The glycerine colored gelatine with fuchsin is applied to the glovebox to make the morphology of the pollens more evident.

Through microscopic analysis it is possible to make a first analysis of the pollen composition of the honey we are analyzing.

DNA extraction

To carry out a more precise analysis, we extracted the DNA from the pollen grains using special DNA extraction kits from plant tissues, in our case the E.Z.N.A. Plant.

Before starting to extract DNA it is important to make sure to dilute the DNA Wash Buffer with 60 ml of 100% ethanol. It is also necessary to prepare two 1.5 ml eppendorf, one containing distilled H2O and the other containing the Elution Buffer, which must be heated to 65 °C.

At this point we can proceed with DNA extraction.

We take 10 g of honey, place it in a 50 ml Falcon and dilute it with distilled water in a total volume of 50 ml. Centrifuge at 15,000 rpm for 15 minutes to precipitate the pollen component.

We remove the liquid being careful not to transport the sediment. Now resuspend the sediment in a 2 ml eppendorf, adding 1 ml of distilled water.

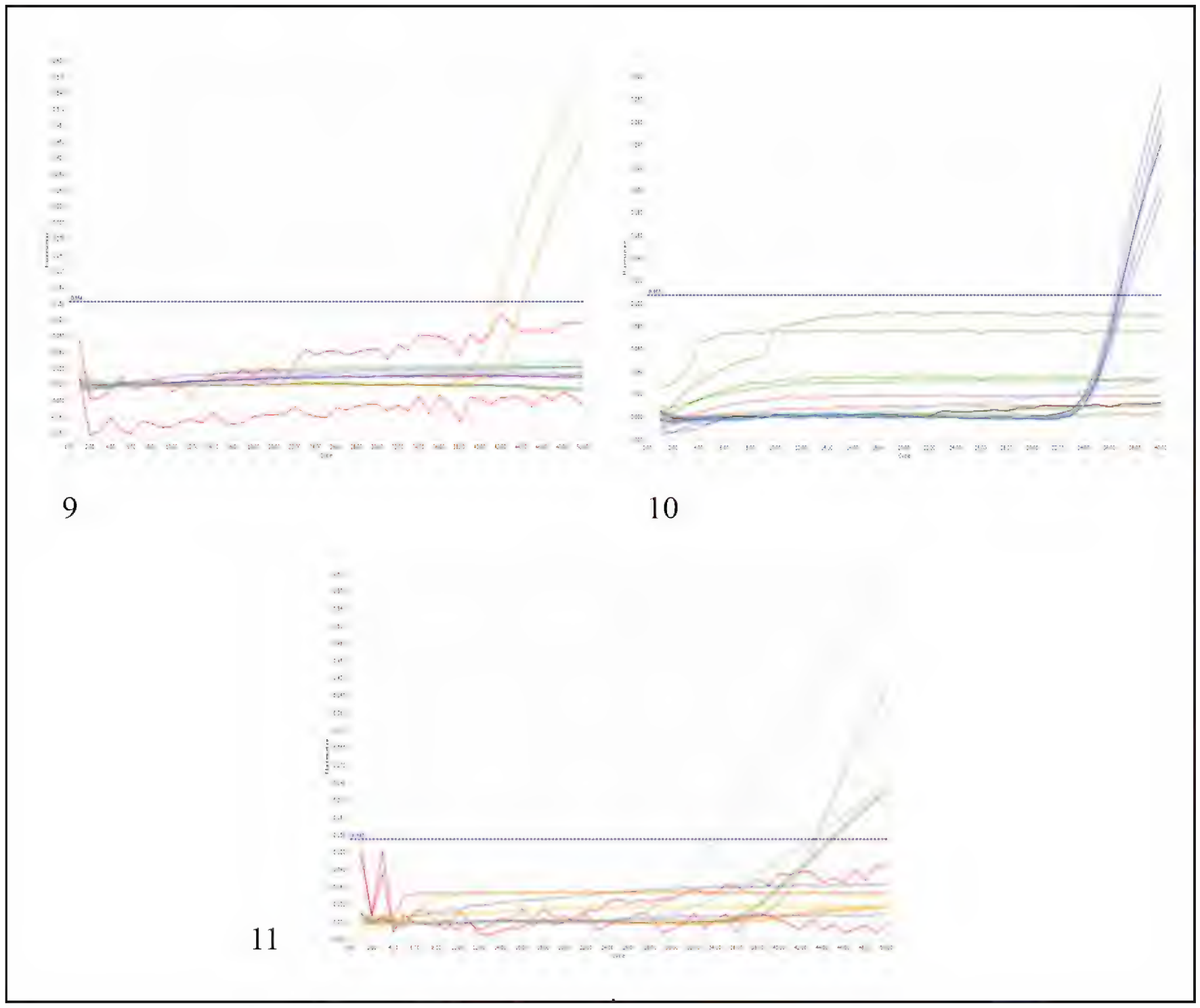
Centrifuge again for 15 minutes at 15,000 rpm. Now we can remove the liquid through a micropipette. In our eppendorf only the sediment containing the pollen grains from which we want to extract the DNA will remain.

Add 600 µl of Buffer P1 to our eppendorf containing the pollen sample and stir it in order to obtain a homogeneous mixture. Incubate at 65 °C for 10 minutes. After incubation, add 140 µl of Buffer P2, stir to homogenate and then centrifuge at 10,000 rpm for 10 minutes.

Transfer all the lysate, taking care not to transfer the insoluble part deposited on the bottom, into a new 1.5 ml eppendorf and add 0.7 volumes of isopropanol, stirring to mix. Centrifuge at 14,000 rpm for 2 minutes to precipitate DNA.

Remove the supernatant, taking care not to transport the sedimented DNA. Add 300 µl of distilled water that we previously heated to 65 °C and 4 µl of RNase A and stir to homogenate.

Add 150 µl of Buffer P3 and 300 µl of 100% ethanol and stir to mix. During this phase precipitates may form. In this case, do "up and down" with the micropipette for 10–15 times in order to homogenate and resuspend the precipitate.



Figures 9–11. Real time PCR on DNA extracted from honeys declared unifloral. DNA extracted from sunflower honey. The only amplified curves are those of the sunflower-specific genes (Fig. 9). DNA extracted from *Eucalyptus* honey. In addition to the specific *Eucalyptus* genes (first two top curves), genes specific to other botanical species have been amplified (Fig. 10). DNA extracted from orange honey. As for *Eucalyptus*, both the specific orange gene and the genes of other botanical species (Fig. 11) have been amplified.

Transfer the entire sample into a column in a 2 ml collection tube and centrifuge for 1 minute at 10,000 rpm.

Remove the collection tube containing the filtrate and use a new one.

Add 650 µl of DNA Wash Buffer into the column and centrifuge at 10,000 rpm for another minute. Remove the filtrate and repeat the previous step again. Centrifuge the column, to dry it completely.

Once dried, we will remove the collection tube and transfer the column to a 1.5 ml eppendorf.

Add 50 µl of Elution Buffer heated to 65 °C and centrifuge for 1 minute at 10,000 rpm.

Repeat this last step without removing the filtrate.

Now we can quantify the extracted DNA to know the concentration of DNA in our sample and its purity.

In Table 1 it is possible to observe the results of DNA quantification.

Real Time Pcr

Real time PCR was performed on the DNA extracted from the pollen grains collected, during the melissopalinological analysis, from honey of eucalyptus, sunflower and citrus declared unifloral.

For each DNA sample to be analyzed, through real time PCR, different mixes are prepared, each containing specific primers for a specific botanical species (Laube et al., 2010; Schievano et al., 2013; Cilia et al., 2018).

Real time PCR was performed in a total volume of 25 µl.

12.5 µl of the TaqMan™ Universal PCR Master Mix, containing the Taq polymerase, magnesium ions, reaction buffer and dNTP, are taken and inserted into a 0.2 ml eppendorf.

Then we will take 0.5 µl of each of the two primers (forward and reverse) and 0.5 µl of the probe and insert them into our eppendorf. Finally add 6 µl of distilled water.

The amplifications were carried out with the following protocol: 56 °C for 3 minutes, 95 °C for 10 minutes followed by 40 cycles of 15 seconds at 95 °C and 45 seconds at 60 °C. Amplification curves are at figures 9, 10, 11.

RESULTS AND DISCUSSION

The results of the molecular analysis, through the use of real time PCR, support the microscopic analysis of the pollens extracted from some commercialized unifloral honeys.

A PCR operative protocol for the species-specific research of particular pollens has been optimized.

The microscopic observation allowed, in fact, to observe the presence of both pollen granules belonging to the species stated in the honey label and to different species.

Since most of the botanical species used for honey production have an entomophilous pollination, the presence of pollen grains belonging to different species, transported by pollinators, is quite logical. However, in order to be able to declare a honey as unifloral, it is necessary that a certain ratio of concentration be observed between the various pollen grains present.

Real-time PCR results show an amplification both in samples that contained the primers of specific genes of the botanical species that characterize the honey analyzed both in samples that contained primers of genes specific to other botanical species (Figs. 9–11).

In the first case there was a greater amplification and after a lower number of cycles, in the second case the amplification was less evident and delayed.

CONCLUSIONS

The use of microscopic and biomolecular analysis techniques applied to melissopalynology allowed to carry out an evaluation on the pollen composition of some honey commercialized and declared unifloral.

Sample	ng/ µl	260/280	260/230
Eucalypt honey	9.34	2.10	0.70
Sunflower honey	9.76	2,17	0,34
Citrus honey	9,55	1,90	0.65
Miele di Sulla	9.97	2.00	0,80
Acacia honey	8.50	1.60	0,60
Dandelion honey	9.60	1,60	0,52
Linden honey	10,86	1,50	0,60
Asphodel honey	5.26	2,83	0,95

Table 1. Concentration and purity values of the DNA extracted from the analyzed honey.

Species	Pollen percentages
Cardus galactites	5% - 25%
Citrus spp.	> 10%
Castanea sativa	> 90%
Eucalyptus botryoides	> 90%
Rosmarinus officinalis	> 10%
Heliantus annuus	15% - 90%
Hedysarum coronarium	> 50%
Taraxacum officinale	5% - 30%

Table 2. Some percentages of pollen grains belonging to the botanical species characterizing honey according to Ente CRA.

The biomolecular techniques can be used to support classical melissopalynology as a complement to the enhancement of products containing pollens.

This type of investigations are particularly important for checking the correct labeling of honey, in particular when the botanical origin is to be labeled.

According to the Legislative Decree n.179 of 21 May 2004, in order to be declared unifloral, the honeys must have very precise chemical - physical characteristics, such as sugar content, water content and content of substances insoluble in water.

There are also established pollen values belonging to the botanical species that characterizes honey, reported in the characterization charts of each honey, which must be respected.

These values, shown in Table 2, can be widely variable or very rigid, depending on the characteristics of the botanical species.

Sunflower honey, as shown in Table 2, can present quite variable values of Sunflower pollen, ranging between 15% and 90% of the total.

In *Eucalyptus* honey, more than 90% of the pollen grains present in honey must belong to *Eucalyptus* flowers.

Although this type of investigation can still be perfected, a PCR operative protocol for species-specific research of particular pollens has been optimized.

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Biodiversity Journal, 2019, 10 (4): 337–342 https://doi.org/10.31396/Biodiv.Jour.2019.10.4.337.342

The last alien reaching Sicily: *Isognomon legumen* (Gmelin, 1791) (Mollusca Bivalvia Isognomonidae)

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ABSTRACT The finding of some living specimens of an alien bivalve of the genus *Isognomon* Lightfoot, 1786 (Mollusca Bivalvia Isognomonidae) attached to rocks is here reported in Sicily for the first time. This is the last of a series of numerous finding of alien molluscs reports in the same area, for which a human-mediated model was supposed. An attempt to indicate the age of the specimens are here furnished on the basis of the number of byssus filaments. Some further environmental notes underline how in recent times these alien species seem better integrated inside the indigenous benthic communities in the Southern Mediterranean coasts, being better allowed in the first settlement by the recent climatic changes and resulting ecologically well organized and structured as in the tropical environments of provenance.

KEY WORDS Alien species; Mediterranean; invasive species; Sicily; Bivalves; Mollusca; *Isognomon*.

Received 13.09.2019; accepted 21.11.2019; published online 11.12.2019

INTRODUCTION

Due to the high increased number of alien species signalled in the Mediterranean Sea, in the last decades the tropicalization in marine environments seem to be a process less ethereal and more real than in the recent past. Findings of non-indigenous mollusc species, like *Pinctada radiata* (Leach, 1814), started just after the Suez Canal aperture (Monterosato, 1878). Nowadays, numerous records concern in particular the eastern regions of the Mediterranean, i.e., Lebanon, Israel, Turkey, Cyprus or Egypt, being the first landing places for newcomer molluscs from the Red Sea. Another different way is the human-mediated invasions, which seem to justify too rapid spreadings of alien species in those regions far from the main Mediterranean entrances. Among the latter, many are due to ballast waters of ships, whose traffic is more increased today (Zibrowius, 1992).

In the Mediterranean Sea two species of *Isognomon* Lightfoot, 1786 (Mollusca Bivalvia Isognomonidae) are reported: *I. legumen* (Gmelin, 1791), which was firstly recorded and reported by Mienis et al. (2016) from Israel, and *I. australica* (Reeve, 1858), reported by Angelidis & Polyzoulis (2018) from Greece only in very recent times.

Despite its recent penetration, *I. legumen* seems on the contrary well distributed in the Mediterranean Sea considering all the official records reported and those which could instead be considered misidentifications with other species, i.e., *Malleus regula* (Forsskål in Niebuhr, 1775) (Bivalvia Malleidae) (see for instances Crocetta, et al., 2017, and Stamouli et al., 2017, Crocetta, 2018).

In Sicily, findings of human-mediated alien species have considerably intensified in these last two decades, with records of species which often have been well established. The last in order of time is *Lottia* sp. (Scuderi & Eernisse, 2016), whose complete identification and origin are still under study.

The discovery of seven living specimens and a shell of the Bivalve *I. legumen* further updates the number of alien species in central Mediterranean, adding new informations on the invasion modalities and possibilities of establishment success of the species inside the basin. This renovate the invocation to pay higher attention to ballast water, whose management in Mediterranean Sea and not Mediterranean harbours should be regulated through stricter lows, which allowed limitations on the spreading of alloctons to different geographic sites.

ABBREVIATIONS AND ACRONYMS. h: height; spec.: living specimens; sh.: shell/shells; st.: station; AGC: Alfio Germanà collection (Catania, Italy); PMC: Pasquale Micali collection (Fano, Pesaro-Urbino, Italy); DSC: Danilo Scuderi collection (Scordia, Catania, Italy).

MATERIAL AND METHODS

The finding of a single specimen of *I. legumen* along the rocky shores of Catania, eastern Sicily (Fig. 1) in August 15, 2017 was rather fortuitous, since it lied unattached on the rocky bottom at -1.5/2 m depth. Probably during the night, fishes feed on the mussel layer and detached it from the rocks. Two thorough samplings lead to the finding of other four spec. in the same season, which were handily removed from the rocky substrates, at -1 to -4 m depth. In summer (July/August) 2018 and 2019 four more specimens were collected. In the same area and conditions in addition a complete sh. was found among shell grit at -2 m depth.

Shells were measured for morphological analysis and the soft body was removed from each specimen's shell and preserved in 90° ethanol for future molecular analysis. Observations were conducted with a stereoscope and photographs and drawings documented the collected specimens.

The Sicilian material was compared to specimens collected in the Persian Gulf (PMC) and to specimens of *I. australica* from Karpatos, Greece,

Figure 1. Map illustrating the collecting locality of *Isognomon legumen* in eastern Sicily (red spot).

Figures 2–7. *Isognomon legumen*. Fig. 2: A specimen in upper view, Catania, eastern Sicily, h: 1.4 mm. Fig. 3, internal side of the left valve. Fig. 4: internal side of the right valve. Fig. 5: byssus. Fig. 6: specimen from Catania in upper view, h: 22.1 mm. Fig. 7: a pearl from a specimen from Catania, h: 1.2 mm. Figure 8. A living specimen in aquarium from Catania, h: 20.2 mm. Figures 9–12. Two specimens from Abu Dhabi, Persian Gulf, in upper and internal view with detail of the ligament, h: 31.2 and 42.0 mm respectively. Fig. 13. *Isognomon australica*, Karpatos, Greece, h: 3.4 mm.

at -38 m depth, found in september/2017 (AGC) for supporting the morphological determination.

RESULTS

Systematics

Classis BIVALVIA Linnaeus, 1758
Ordo OSTREIDA Férussac, 1822
Superfamilia PTERIOIDEA Gray, 1847
Familia ISOGNOMONIDAE Woodring, 1925
Genus *Isognomon* Lightfoot, 1786
Type species: *Isognomon perna* (Linnaeus, 1767)

Isognomon legumen (Gmelin, 1791) (Figs. 2–12)

EXAMINED MATERIAL. Italy, Catania, eastern coast of Sicily, 9 spec. and 1 sh. along the northern rocky shore of the city, -1/4 m depth (DSC). Four sh. of *I. legumen* from Abu Dhabi, United Arab Emirates (UAE), Persian Gulf, -3/6 m (PMC).

DESCRIPTION. Specimens collected were assigned to *Isognomon* on the basis of the characteristic "denticulate" ligament, being the hinge straight and edentulous (Figs. 3, 4 and 10–12). The height of specimens varies from 22.6 to 14.8 mm. Valves (Figs. 2–12) are variable in form, relatively solid and moderately convex, rounded at margins except the upper side, which is straight, with a pointed laterally placed umbo; they are ornamented by marked and fragile laminate processes, smooth and nacreous on the internal side (Figs. 2, 4 and 10, 11) and white-greenish in colour on the external side, with orange/yellowish periostracum.

Soft parts (Figs. 4 and 8) are pale green-yellowish with orange edges and gill tips of the mantle; gonad is almost egg-yellow. Byssus is constituted by numerous not very long green filaments (Fig. 5).

A single drop-shaped olive-green pearl (Fig. 7) 1.2 mm high has been found inside a specimen.

Specimens from the Persian Gulf (Figs. 9–12) were thicker and higher, reaching 40.22 mm, but substantially with the same morphological characteristics.

DISTRIBUTION AND BIOLOGY. Specimens collected in eastern Sicily were attached with the byssus to epibionts living on the volcanic substratum, among the trottoir constituted by other molluscs, many of which were alien too, and red calcareous algae present in the subtidal zone. Other species present were: *Brachidontes pharaonis* (P. Fischer, 1870), *Pinctada radiata* (Leach, 1814), *Ostrea stentina* Payraudeau, 1826, *Anomia ephippium* Linnaeus, 1758, *Mytylus galloprovincialis* Lamarck, 1819, *Aplysia dactylomela* Rang, 1828.

Apart the first almost fortuitus record, among the algal turf and between the other epibionts present and constituting a trottoir, specimens live collected were easily detectable by the yellowish mantle seen between opened valves.

REMARKS. *Isognomon legumen* was often misidentified with *Malleus regula* (Crocetta, 2018), from which could be easily distinguished by the edentuous hinge in which 5–6 peculiar resilifer bearing the ligament are present (Fig. 12). Being recently penetrated in the Mediterranean, *I australica* (Fig. 13) is the most similar species in this basin. This latter has flat, almost smooth, except for some faint and flat axial ridges, fragile and semitransparent valves, which make the species distinguishable from *I. legumen*. Moreover, the niche they prefer is different: specimens of *I. australica* were collected attached with byssus under stones in the upper infralittoral zone, preferring a slightly deeper fringe (Angelidis & Polyzoulis, 2018 and Micali pers. comm. feb/2019).

DISCUSSION AND CONCLUSIONS

Harper & Morton (1994) reported the species reaching a probable age of a couple of years, which is therefore quick in growing, the age being probably correlated to the number of filaments of the byssus as suggested by the same Authors. Sexual maturity seem reached just in specimens of 6 mm (Harper & Morton, 1994): so all the Sicilian specimens were mature. They reported the most frequent height of studied material around 50 mm (Harper & Morton, 1994, fig. 6), while an adult specimen of 14.4 mm showed a byssus constituted by 42 filaments. The maximum height reached by specimens here studied was 22.6 mm, with a cone of byssus constituted by almost 150 filaments,

thus resulting almost full grown, though smaller in dimensions, and probably settled two years before.

This observations place chronologically the Sicilian record approximately at the same time with that of Israel (Mienis et al., 2016), which is the first for the Mediterranean Sea. Putting all these facts together, we can say that, while the Israeli population of *I. legumen* probably arrived from the Suez Canal, a human-mediated mode of invasion of the Sicilian specimens is the most probable explanation.

In Stamouli et al. (2017), Ovalis and Zenetos reported the finding of fourteen specimens of *I. legumen* in Dalyan, Iztuzu, and southern Turkey, but the specimen in figure 20, p. 548, is instead *I. australica*. Judging by the reported geographical distribution Micali et al. (2017) consider *I. legumen* and *I. australica* as synonyms.

Some final considerations should be done on the ecological conditions in which *I. legumen* was found in Sicily. In fact, the structure of the biofouling community living on a *Pinctada* bed in the Red Sea described by Wronski (2010) are similar to that of the Sicilian hard substrate community found in some locality. In particular, actual chemical-fisical parameters in terms of water (surface temperature, salinity) in eastern Sicily during the late spring and summer seasons (pers. obs.) are similar to those reported by this last Author for the Red Sea, although high seasonal excursions persist in Mediterranean temperate localities. Once entered the Mediterranean, the same species seem to aggregate together to maintain the same structure of tropical community. Surprisingly, as personally observed, some species like *Pinctada radiata*, long time after the first invasion of a certain area, tend to form a more complex structure of numerous specimens attached to each other and to aliens of different species merging together and forming tropical-like enclaves similar in structure to those described for the Red Sea (Wronski, 2010). These "pseudo-tropical" structures seem subjected to annual fluctuations and in some years they appear less structured than in other years.

The regular finding of specimens of *I. legumen*, though never abundant, during these last three years seem to confirm the consolidation of the Sicilian population of this species.

ACKNOWLEDGENENTS

This research was sponsored by the 'Città Metropolitana di Messina' the official management body of the reserve (permission no. 557/VIII DIR, 12.03.2014). Facilities were provided by the mussel farm FARAU s.r.l.

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Biodiversity Journal, 2019, 10 (4): 343–352 https://doi.org/10.31396/Biodiv.Jour.2019.10.4.343.352

The benthic stands of the soft and rocky substrates of the Paloma Island, Algeria

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ABSTRACT The Oran coastal zone (Algeria) is distinguished by a remarkable wealth of habitat and species (*Posidonia* meadow, coralligenous groupers, and endemic seaweed). This work has allowed us to inventory and identify one hundred (100) macrobenthic species of soft substrates and 65 macrobenthic species of rocky substrates. The contents of the different grain size fractions show a homogeneity between the four stations with a trimodal sand dominated by coarse sand (diameter = 0.08–10 mm). The distribution of these species was mapped using the System of Geographic Information (GIS). Finally, the computation of the Shannon-Wiener (H) and Simpson (Si) indices of the rocky and soft substrates of this study area reveal an average ecological richness with a high biological diversity. It is necessary to undertake annual monitoring of macrobenthos through a wider investigation of several sites and stations in a longitudinal and vertical distribution. The "overall biological quality" index will be calculated and refined in order to rank the different sites by establishing specific indices for each site.

KEY WORDS Granulometry; indice; macrobenthos; mapping; Paloma Island; rocky substrates; soft substrates.

Received 13.07.2019; accepted 25.10.2019; published online 11.12.2019

INTRODUCTION

The Convention on Biological Diversity (https://www.cbd.int/) defines biological diversity or biodiversity as "*the variability of living organisms from all sources including, inter alia, terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part, this includes diversity within and between species and ecosystems. In other words, biodiversity is the variety of life on earth at all levels, from genes to global populations of the same species, from species communities sharing the same small habitat area to global ecosystems*".

This biodiversity is not only a condition for the healthy and natural development of species and ecosystems, but also a natural legacy we leave to future generations. As such, our company has an ethical and moral responsibility.

Marine biodiversity research is timely and fundamental for many reasons. Marine biodiversity plays a key role in the supply and regulation of ecosystem services, provides economic wealth with resources for active substances in pharmacology and the medical field, for fisheries and aquaculture products, but also in the field of cultural well-being, or as a source of relevant models in basic and applied research.

Overall, there is a real lack of synthetic data on national marine biodiversity. When this data is advanced (Algeria, Tunisia for some taxa), it is certainly largely underestimated because resulting from some local studies. In North Africa, entire portions of coastlines and habitats known to harbor high biodiversity have not been explored at all (5ème Rapport National de l'Algérie, 2014).

Without a minimum knowledge of the distribution of characteristic habitats of the Mediterranean Sea, and at least of species with special status (species of interest for conservation), it seems at least complicated to provide tools for assessing the impact of change. There is no real device for continuous observation and biodiversity inventories are often very punctual in space and time (Unep, 2011).

In Algeria, the first observation made is that of a lack of knowledge of a certain component of marine biodiversity. Many taxonomic groups or some benthic communities have not been studied. This action is recommended for all "Environmental quality monitoring networks", in order to have a long-term follow-up and to evaluate the consequences of natural modifications and anthropogenic changes in the environment.

MATERIAL AND METHODS

Study area

The study area on the Plane or Paloma Island (Fig. 1) is located about 7 km from the beach of Bousfer, in the bay of Andalusia, at 35°46.281'N and 0°54.115'W, between Cape Falcon and Lindless cape. The island covers an area of 300 meters long and 100 meters wide. It is uninhabited (except for a gull colony) and has a small dock for small boats. The island has an unoccupied lighthouse in operation. Concretely our sampling area on Paloma Island covers four stations corresponding to the four GPS points whose coordinates are shown in figure 1.

Sampling and treatments of soft and rocky substrates

The sampling of soft sediments was carried in

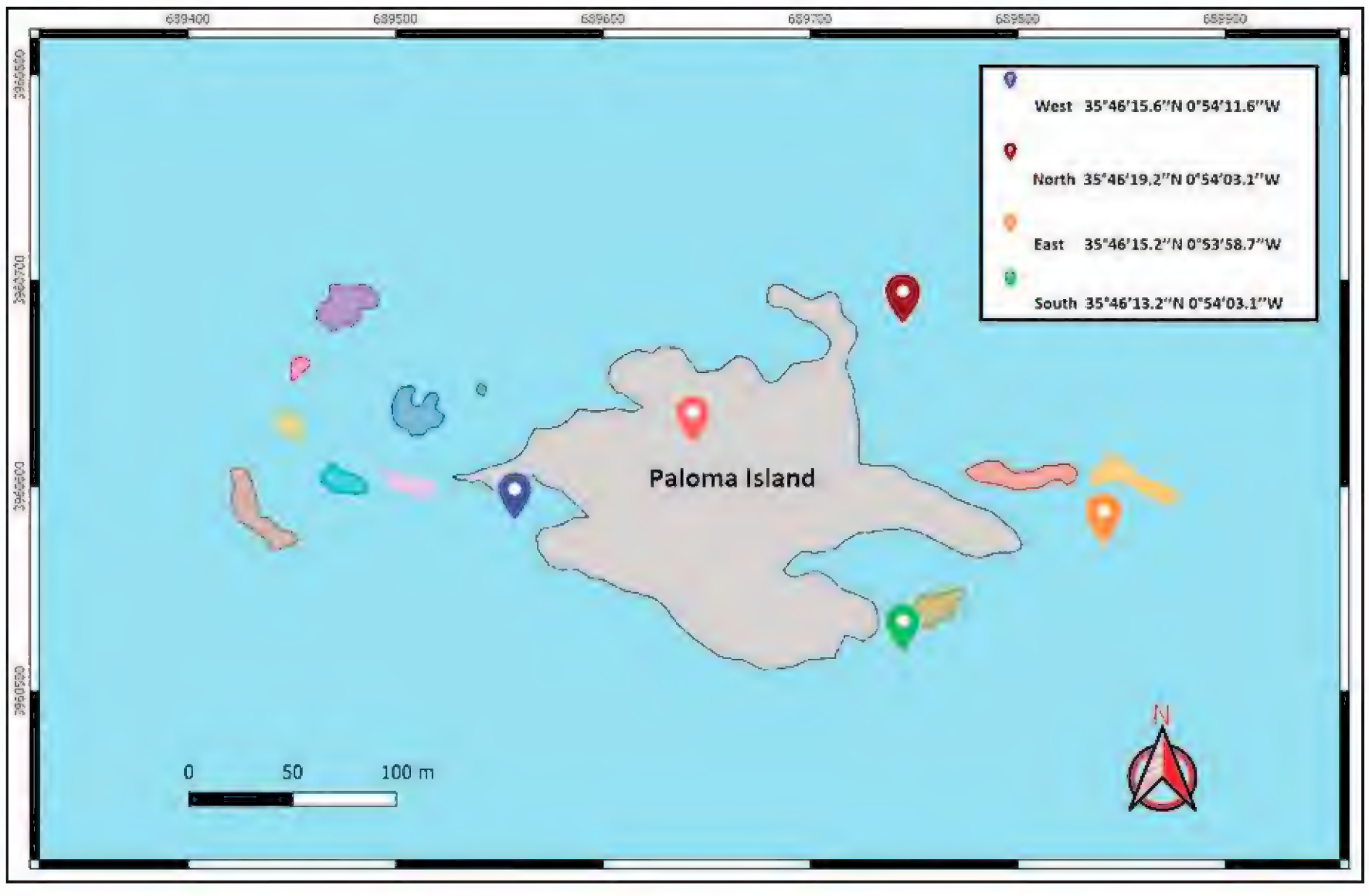


Figure 1. Sampling stations of Paloma Island, Algeria.

December 2018 with a manual grab. The grab shots are replicated four times to account for the distribution of species at a given point and the dispersal of larger species. Following the standard protocol, an extra bucket per station should be collected to analyze the sediments associated with the organisms (granulometric study). The same protocol is performed at the four stations. For rocky substrates a non-destructive sampling was performed. In the laboratory, samples stored in a cooler will be pretreated to remove debris and waste. Depending on their sedimentary nature, the samples are sieved on a 1 mm sieve (round mesh) for fine or silted sediments or 2 mm for coarse sediments. Sieve rejects fixed with diluted formalin (5%) will be subject to biological sorting and identification.

Granulometric study

The study of the soft sediments of the four stations (North, South, East, West) of Paloma Island will be the subject of a granulometric study according to the Norms (NFP 94 056 and 94 057).

Identification of benthos

To be taken into account, specimens must have enough intact parts for correct taxonomic identification, especially having their head (to avoid counting the head and body of the same individual as two animals). It is recommended to use optical equipment (binocular loupe, optical microscope). Larval exuvia or empty shells (e.g., gastropods and bivalves), empty sheaths (e.g., phryganes) are not counted. Subsamples have been sorted until all benthos have been removed. The sorted specimens are subsequently photographed and then re-stored in formalin. Genus and species identification will be done later using identification keys (http://www.marinespecies.org).

RESULTS AND DISCUSSION

Granulometry

The study of soft substrats of Paloma Island has, on the one hand, characterized the sediment cover within the sampling site and, on the other hand, established the spatial variations of these characters between the four stations of the studied site. The contents of the various size fractions show a notable homogeneity. For all the stations (Figs. 2–5) a trimodal sand dominated by coarse sand with main modes of 0.08 mm to 10 mm was found. The general appearance of the all cumulative grain size curves of the analyzed samples is rectified, which reflects the homogeneity of the sediments belonging to the four sampling stations. On the whole, the curves are relatively sloping, which underlines the good classification of the sediments. The presence of coarse sands can be explained by the nature of the study area which is a rocky island. Fine sands are usually found in shallow or coastal areas.

Identification of benthos

The results obtained are only partial and therefore not really representative of the benthic richness of the site. These results show the presence of at least a global number of 100 species collected on soft substrates (Fig. 6), divided into seven groups namely: gastropods, bivalves, echinoderms, crustaceans, polychaete annelids, cnidarians, marine plants and 65 species harvested from rocky substrates (Fig. 7) divided into ten groups namely: gastropods, bivalves, echinoderms, crustaceans, polychaete annelids, polyplacophores, cnidarians, algae, sponges, marine plants.

Classification of species according to their pollution sensitivity

Accidental or recurrent release of a disturbing agent into the marine environment immediately results in physical and chemical changes in water properties as well as short, medium and long-term effects on biota. To obtain information on this type of effect on marine organisms, plankton is not the most useful marine biota compartment at the local level because it is composed mainly of small organisms whose life cycle is short, floating passively in the current (it does not stay in the affected area) and has a high ability to rebuild populations (high reproductive capacity). Necton is composed of organisms (mainly fish) that are very mobile, allowing them to flee and move in search of better conditions. The benthos is the marine biota compartment that offers the most interesting

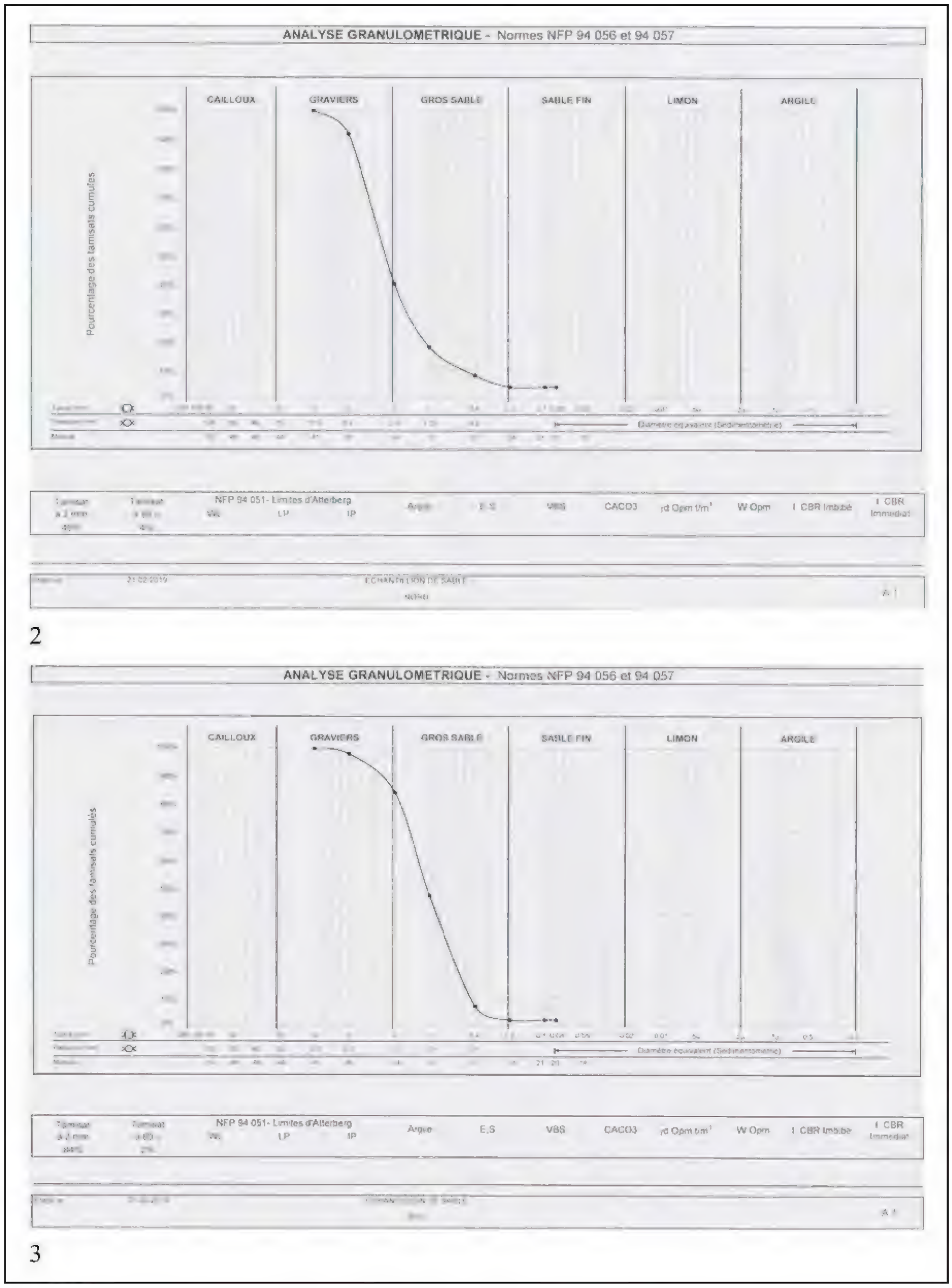
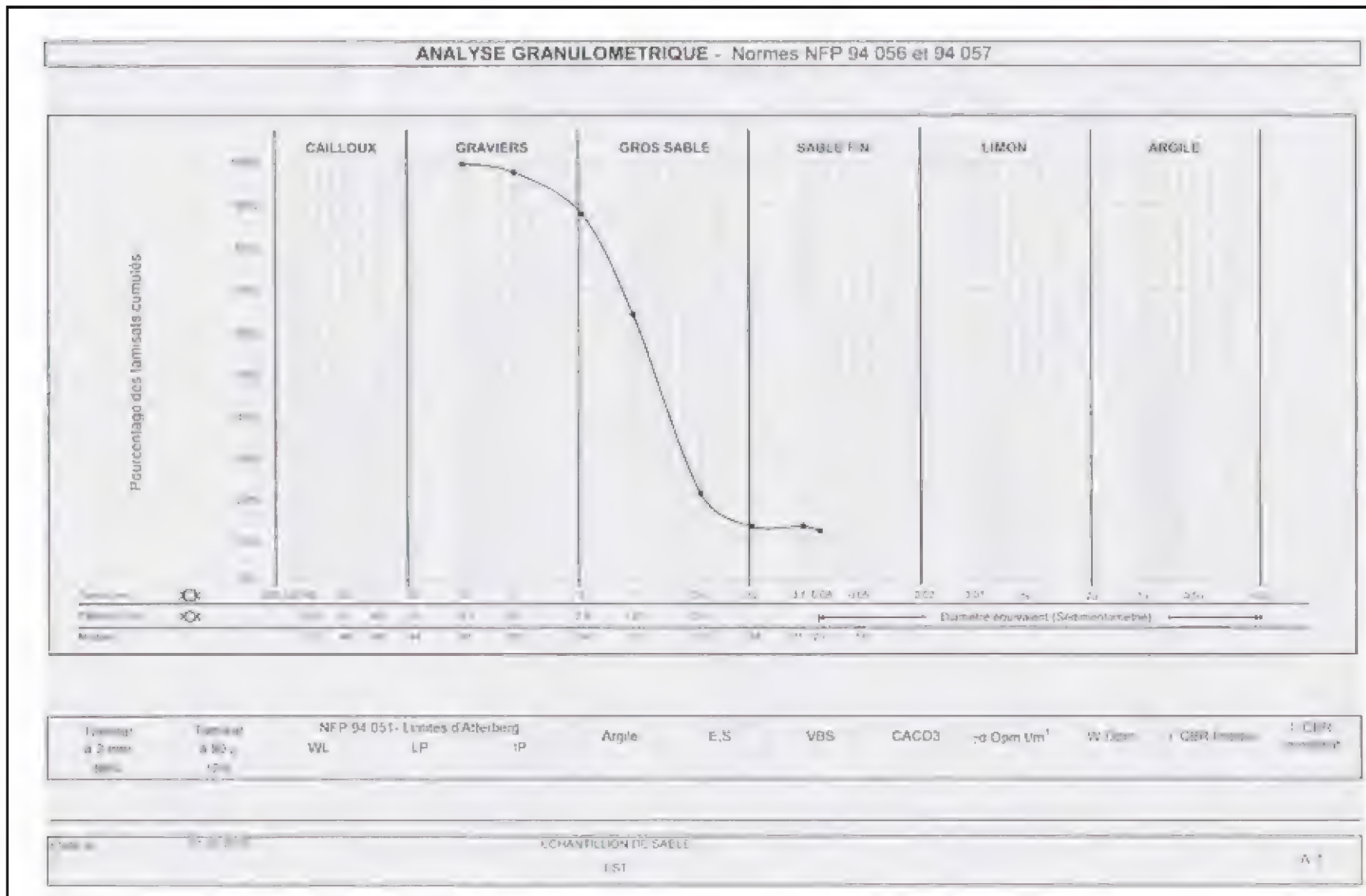


Figure 2. Grain size curve of Paloma Island, Algeria: North station (2019).
Figure 3. Grain size curve of Paloma Island, Algeria: South station (2019).



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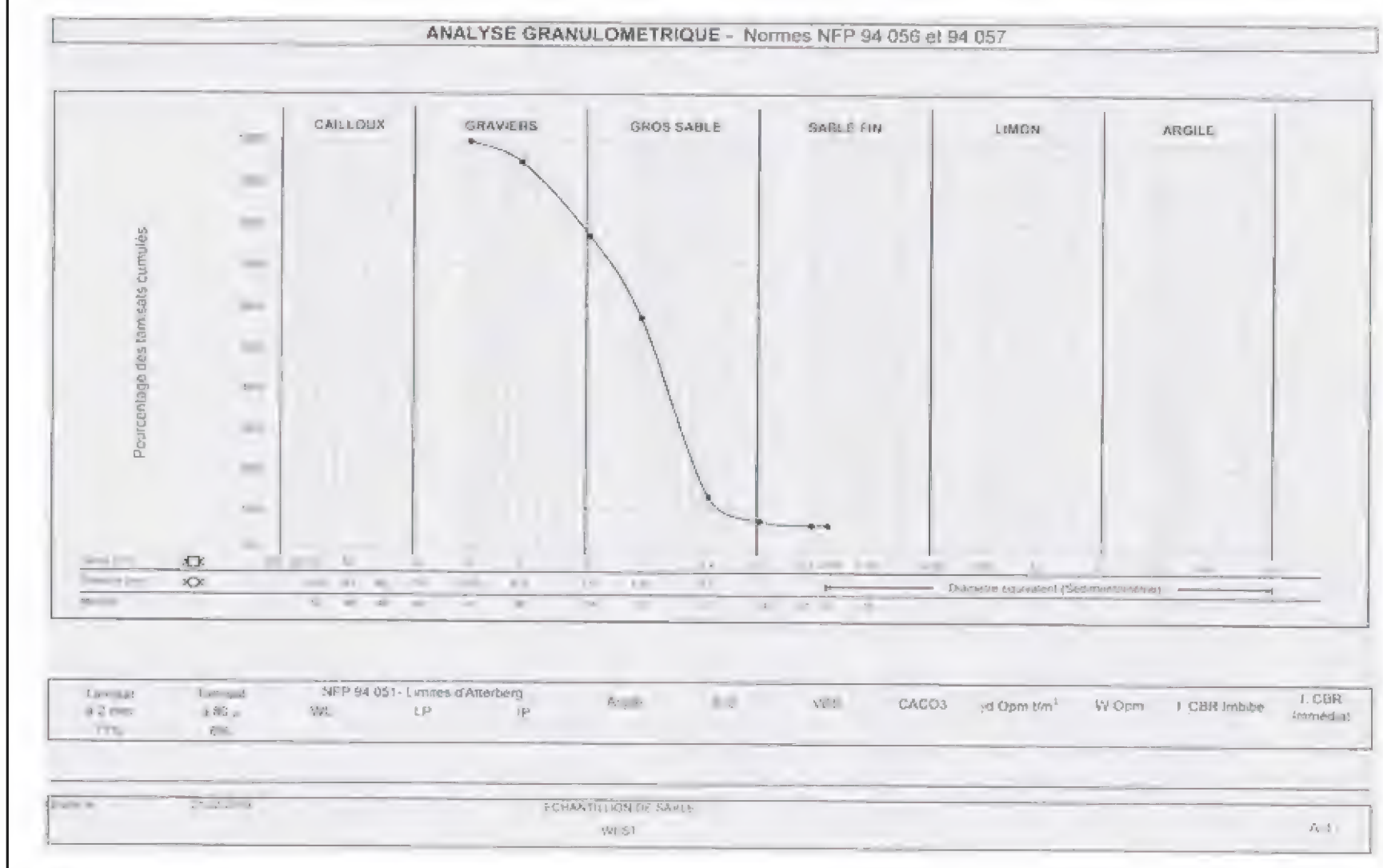

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Figure 4. Grain size curve of Paloma Island, Algeria: East station (2019).
Figure 5. Grain size curve of Paloma Island, Algeria: West station (2019).

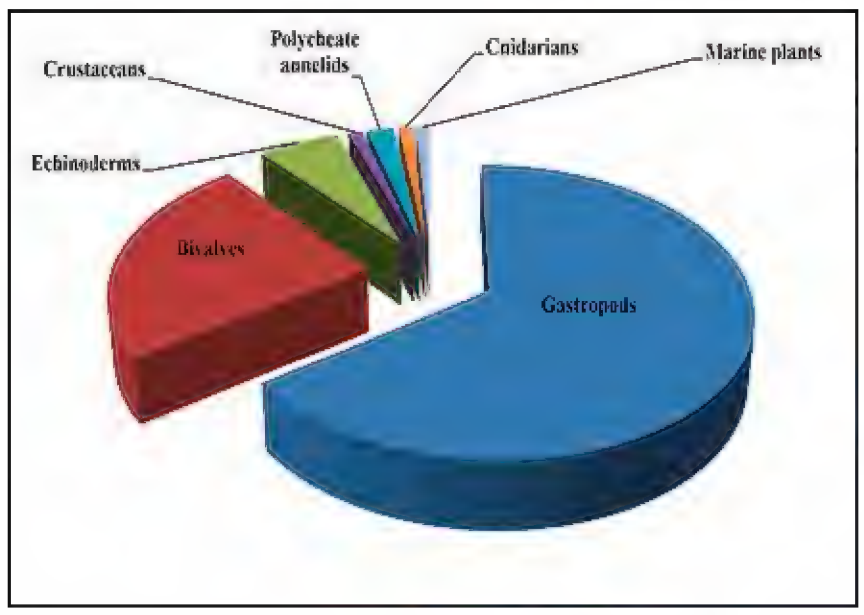

Figure 6. Benthic groups of soft substrates of Paloma Island, Algeria.

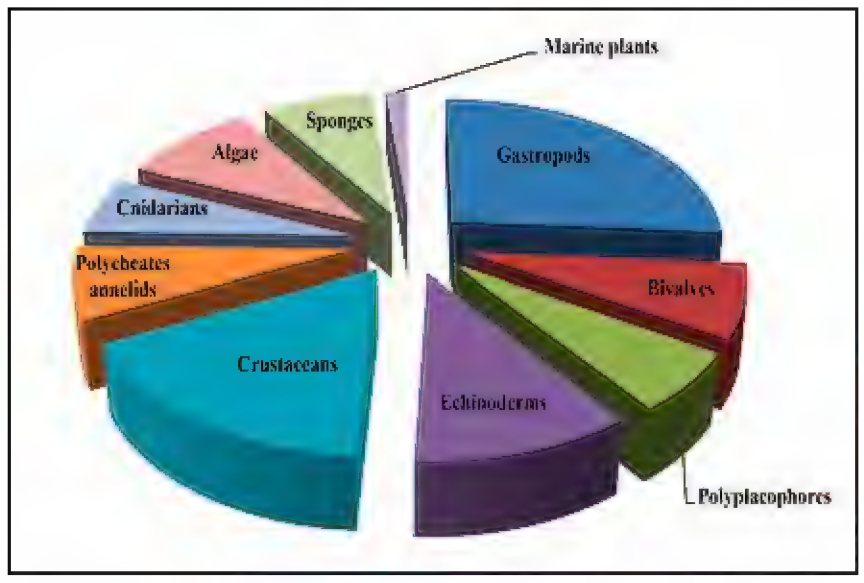

Figure 7. Benthic groups of rocky substrates of Paloma Island, Algeria.

information, mostly sedentary, the benthic organisms remain in the affected area and are unable to avoid the environmental changes likely to occur in their habitat. Therefore, either they survive these changes perfectly or they survive with obvious signs of stress and downsizing or they completely disappear from the system. They can therefore be used as bioindicators (sublethal and lethal effects), as permanent or periodic "sentinels" for the level of quality of the waters in which they live, and contribute greatly to the environmental monitoring of coastal waters (Garcias-Gomez, 2015).

Soft bottoms, more unstable and uniform than rocky bottoms, offer less epibenthic diversity. However, it is on these sea bottoms that seagrass meadows are established, which must be preserved as it plays a major ecological role on our coasts. Especially *Posidonia oceanica* (L.) Delile and *Cymodocea nodosa* (Ucria) Asch. (1870) perfectly visible, localizable and, in general, sensitive to disturbances and pollution. We can observe several types of response to different environmental disturbances. Some of them disappear or decrease massively (sensitive species), others manage to survive normally without showing any external sign of degradation (tolerant species), others still do not decrease but show morphological abnormalities (decay, weak growth, diseases due to microorganisms, etc.) (Garcias-Gomez, 2015).

Several indifferent species for the pollution of soft and rocky substrates have been inventoried (gastropods, bivalves, crustaceans and annelids). Their environmental tolerance gives them a broad ecological valence (Becerro et al., 1994; García-Gómez, 2007). If found in clear waters and rich biodiversity in excellent conservation status, they also support situations of high environmental stress, high turbidity, high sedimentation and moderate levels of suspended organic matter. The great adaptability of these organisms does not make them good indicators of clean and limpid water, as the information provided is very limited. They are a perfect example of species not to be selected for monitoring the evolution of well-preserved funds that may be subject to environmental monitoring through sensitive bioindicators (Carballo & Naranjo, 2002).

Among the species sensitive to the pollution of soft and rocky substrates there are the *Patella* Linnaeus, 1758 (Gastropoda Patellidae), particularly *P. ferruginea* Gmelin, 1791. This species can not be considered strictly sessile because it is vagile at high tide, it then moves actively around its substratum where it returns to settle at the rock at low tide. In addition, it is not a FBI species (Fixed Biological Indicator) and according to the design of Rovere et al. (2015), it can not be considered as such at low tide. Endemic species of the western Mediterranean it is in marked decline since the beginning of the 20th century. It has almost disappeared from continental European coasts where it is now reduced to small subpopulations in Corsica, Sardinia and the island of Pantelleria. On the African coast, its presence is restricted to Morocco, Habibas Islands in Algeria, Zembra island and Cap Bon in Tunisia. In Spain, there are some colonies in Andalusia and in the region of Murcia, the main

ones being in Ceuta, Melilla and in the Chafarinas Islands. The *Patella* are species traditionally associated with limpid and well oxygenated waters. They are sensitive to pollution, turbidity and oxygen depletion in water (Espinosa, 2005). In several studies, they have been proposed as species indicative of good environmental conditions (Espinosa et al., 2007).

The emblematic species of the Mediterranean Sea widely used in the biomonitoring of soft and rocky substrates is *Posidonia oceanica*.

According to the clarity of the water, it is found from the surface up to 40 meters deep. This Mediterranean endemic species is indicative of clean, unpolluted and well-oxygenated waters (García-Gómez 2007, Montefalcone 2009, López & Royo et al., 2011). It is particularly sensitive to the progressive increase in turbidity, especially if it is associated with an organic load (Cancemi et al., 2003) and a high sedimentation rate (Ruiz & Romero, 2003, Sánchez-Lizaso, 2004). This species is one of the most emblematic of the Mediterranean Sea for monitoring and environmental monitoring, due to the sensitivity of seagrasses to anthropogenic disturbances. Figures for protection Inscribed in Council Directive 92/43/EEC (Annex I: Types of natural habitats of Community interest whose conservation requires special conservation areas) Listed in the Spanish and Andalusian Lists of Wild Species special protection scheme (LESRPE, Royal Decree 139-2011 and LAESRPE, Decree 23/2012).

Statistical descriptors

They are the basis for calculating many other

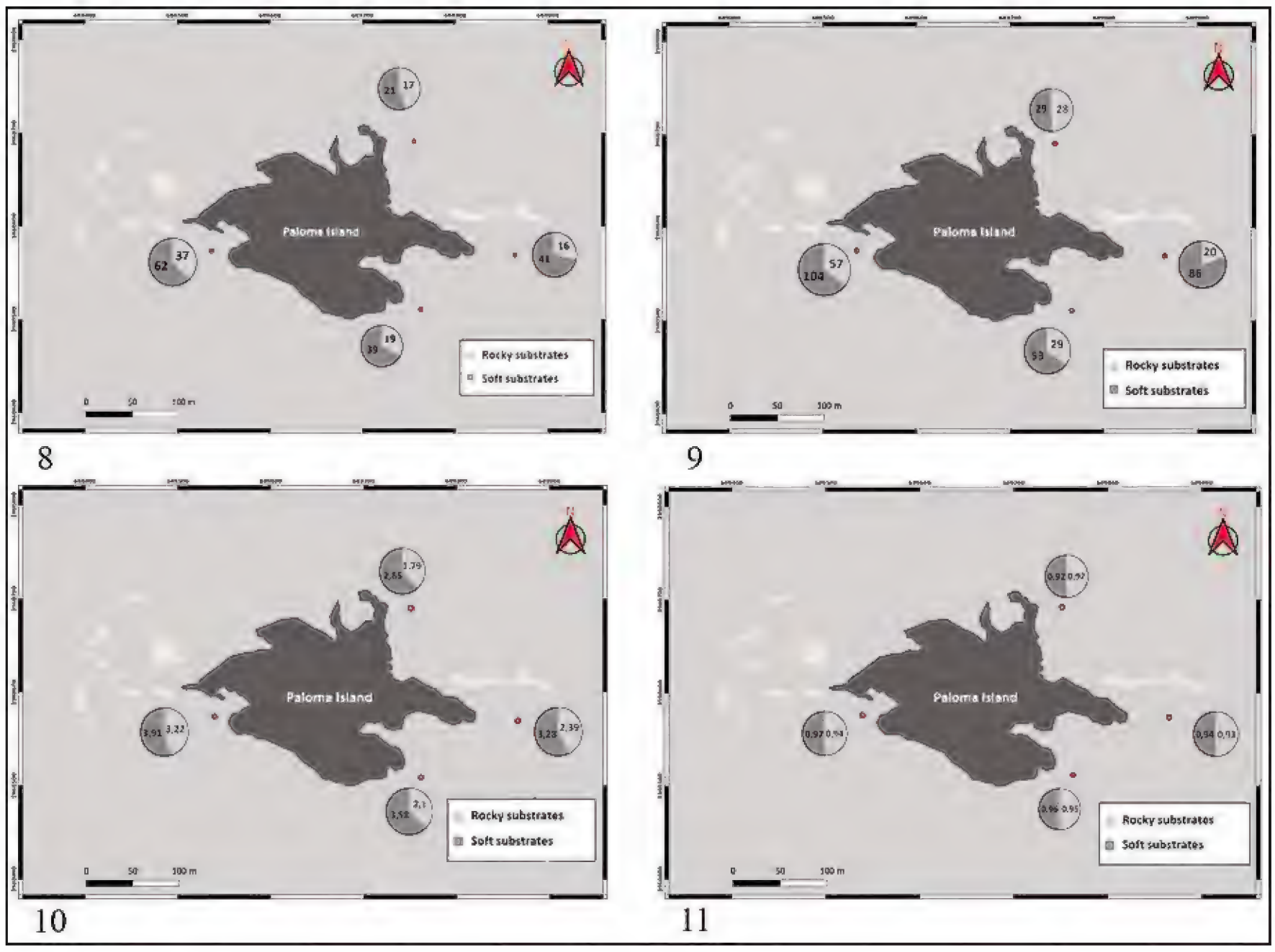

Figure 8. Specific wealth of soft and rocky substrates of Paloma Island (Algeria). Figure 9. Abundance of soft and rocky substrates of the four Paloma Island stations (Algeria). Figure 10. Shannon-Wiener index of the soft and rocky substrates of the four Paloma Island stations (Algeria). Figure 11. Simpson's index of soft and rocky substrates at the four Paloma Island stations (Algeria).

more complex indices. However, they are often influenced by the sampling method, sample size, and identification procedures: the site can not be sampled in its entirety, the number of species present in the samples do not generally reflect the absolute diversity but the apparent diversity. In addition, these methods do not take into account the relative abundance of each species, which contributes to the diversity of the site. The validity of these descriptors on communities growing on hard substrates is questionable because of the difficulty in counting colonial organisms, or the difficulty of sampling. Thus, the values of these indices can only be compared when the sampling protocol has been the same. In addition, these indices are highly dependent on habitat type, and the determination of average values representative of a state of environmental quality requires the determination of threshold values for each habitat type (Grall & Coïc, 2005).

Specific wealth

This map represents the specific richness of the soft and rocky substrates of the four Paloma Island sampling stations, on a scale of 1/50 m. The specific richness of soft substrates is greater than that of rocky substrates (Fig. 8). The spatial variation of this same index shows that the western sector represents the most important specific richness, it is supposed that this is due to the presence of several islets which offer a multitude of habitats and refuges to the benthic species. The species richness index has the drawback of being strongly dependent on the size of the samples (the number of species sampled increasing with the sampled surface) and the type of habitat (the specific richness varies according to the type of substrate, depth, salinity, etc.). It remains difficult to make a descriptor of the state of a medium. Simboura & Zenetos (2002), however, suggest assigning threshold values for different types of ecological groups and for different habitats.

Abundance

In the face of pollution, species will follow three types of reaction according to their sensitivity:

-Disappear, for the most sensitive.

-To maintain oneself, for the indifferent ones.

-Take advantage of the new conditions put in place and develop, for the tolerant and opportunistic.

These different responses will translate into the abundance of species. Abundance profiles over time are therefore widely used as indicators of the effects of pollutants in sediments, as well as biomass and species richness curves (Grall & Coïc, 2005).

This map represents the abundance of soft and rocky substrates at the four sampling sites on Paloma Island. Abundance of soft substrates is shown to be greater than the abundance of rocky substrates (Fig. 9). Similarly, the species richness of the benthic species is greater at the western station. The softer substrates, which are easier to sample with four-fold replica grab, have made the number of species more important, although it is known that it is rather the rocky substrates that offer many habitats and shelters to them. In addition, plant species are difficult to translate in terms of numbers, and their biomass can not be quantified.

Shannon-Wiener (H) index

The Shannon-Wiene index (H) is calculated just for fauna.

We have considered for comparison the scale proposed by Simboura & Zenetos (2002), for sandy/muddy habitats:

The index H':> 4 is very balanced; 2.5-4 balanced; 1.5-2.5 unbalanced and <1.5 very unbalanced.

This map represents the Shannon-Wiener index of soft and rocky substrates at the four sampling sites on Paloma Island (Fig. 10). According to the stand classification thresholds from the Shannon H index. The ecological state is average (balanced), and the classification of the pollution is moderately polluted for the rocky substrates. Even though the island is classified marine protected area, it should be noted that the levels of protection of these marine areas is derisory. The marine environment in general, and the Algerian coast in particular, constitute a rich sea sheltering ecosystems, which, in recent years, have been markedly degraded, mainly due to pollution and human impact, including shoreline developments, overfishing, trawling, the introduction of exotic species that can lead in the

near future to a trivialization of stands and ecosystems.

Simpson index (Si)

The Simpson index (H) is calculated just for fauna.

Simpson's index of soft and rocky substrates tends to 1, reflecting a high diversity (Fig. 11). The homogeneity of this index between the two types of substrates depends on the fact that these species share the same distribution areas, and therefore even if the workforce is not very important because of the Mediterranean is a poor enough sea, in terms of nutrients, it offers a very important diversity, particularly related to the Messinian crisis.

CONCLUSIONS

The objective of this study is to demonstrate the value of an ecosystem approach to assess the quality of the marine environment. The proposed approach is therefore global, based on the study of Benthos quality of soft and rocky substrates. The ecosystem approach proves to be highly sensitive, as it differs from the ecotoxicological approach and is non-discriminatory with respect to pollutants that require costly measures and thus allows harmonization of interpretations. It also makes it possible to compare the sites with each other in terms of benthic richness and to provide elements for classifying areas or reviewing the status of certain species or inventories of new invasive species.

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Biodiversity Journal, 2019, 10 (4): 353–358 https://doi.org/10.31396/Biodiv.Jour.2019.10.4.353.358

Some parameters of growth, mortality and exploitation rate of round sardinella, *Sardinella aurita* Valencienne, 1847 (Pisces Clupeidae), fished in Oran bay (Algeria)

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ABSTRACT Growth and mortality parameters of *Sardinella aurita* Valencienne, 1847 (Pisces Clupeidae) were estimated based on length frequency distribution data. A total of 894 sardinella were collected between march 2008 and March 2009, from Oran bay (Algeria). The parameter b in the present study (W = a. Ltb) is 3.1 and indicates that *S. aurita* had major allometric growth. The von Bertalanffy growth parameters were estimated by using the software FISAT II and showed that the asymptotic length (L ∞) is = 34.21 cm for females and 33.68 cm for males. The growth coefficient (K) is 0.47(1/year) for females and 0.39(1/year) for males. The value of total instantaneous rate of mortality (Z) is 2.41 and the natural mortality rate (M) is 0.79. The fishing mortality was obtained by F=Z - M = 1.62 year -1. The exploitation rate (E) is 0.67 year -1 and indicates that the stock of *S. aurita* from Oran waters is in overexploitation state.

KEY WORDS Bay of Oran; exploitation; growth; mortality; *Sardinella aurita*.

Received 24.07.2019; accepted 25.10.2019; published online 11.12.2019

INTRODUCTION

The round sardinella, *Sardinella aurita* Valencienne, 1847 (Pisces Clupeidae), is a marine pelagic fish that is widely distributed throughout tropical and subtropical seas including the entire Mediterranean Sea and Black Sea (Froese & Pauly, 2000). Clupeidae are key species in the marine food chain and their presence is needed to maintain the balance of ecosystems (Smith et al., 2011). Sardine fishery is one of the most important fisheries worldwide and one of the most important species of Algerian fisheries. The *S. aurita* is a very common and frequent fish in Algerian coasts (Djabali et al., 1993).

The aim of the present study is to estimate the following parameters: growth, mortality and exploitation rate, which are required for assessing and managing the stock of *S. aurita.*

MATERIAL AND METHODS

Sampling

A total of 894 specimens of *S. aurita* were collected monthly during the period from March 2008 to March 2009, from the commercial catch of Oran coast. Our study area is located in the north-west of Algeria and south-west of the Mediterranean Sea (Fig. 1). The port of Oran is situated at the bottom of gulf, between the tip of the Canastel and Cape Falcon northwest of Ain el Turk (Kerfouf et al., 2010; Keddar et al., 2016).

The total length (TL, in centimeters) and the total weight (TW, in grams) for each specimen were measured. The length frequency distributions were arranged in 1.0 cm intervals. A ichtyometer of 50 cm has been used to determine the size of the fish. When assessing the metric characters, the standard methods of FAO have been applied. The sexes were determined by macroscopic observation of the gonads.

The length frequency distribution of the species was represented by percentage length frequency at intervals of 1 cm. The length frequency data analysis was made to estimate the Von Bertalanffy growth model represented as $Lt = L\infty (1 - exp\text{-}k (t\text{-}to)$. Where Lt is the average predicted length at time t, $L\infty$ is the hypothetical asymptotic length, K the growth coefficient, t0 the hypothetical time at which fish length equals 0 and t is the age. The growth parameters such as K, $L\infty$ and t0 were estimated by the method ELEFFAN using the FISAT II.

Length-weight relationships

The length-weight relationships were determined according to the equation: WT = aLTb; WT is the total body weight (g), LTis the total length (cm), while "a" is constant and "b" is length exponent. The "a" and "b" and "r" values were calculated from linear regression of the fish length and weight measurements, which we express as: Log W= Log a + b Log Lt. The relation is isometric when b=3. If b<3 the allometry is positive and when b>3 the allometry is negative.

Figure 1. Study area: Oran bay, Algeria.

Fish mortality and exploitation ratio

Determining mortality rates is critical for determining abundance of fish populations. Using the model Z=M+F with M being Natural mortality and F being Fishing mortality, the natural mortality was calculated using Pauly's empirical equation (1980). It assumes that there is a relationship between size and natural mortality. Pauly's method was based on the correlation of M with von Bertalanffy growth parameters (K and $L\infty$) and temperature (Gunderson, 2002): Log M = - 0.0066 – 0.279. Log $L\infty$ + 0.6543. Log K + 0.4634. Log T°. Where: $L\infty$ = asymptotic length, K = growth coefficient, T = average annual temperature of the stock's habitat, in °C, considered at 18 °C for *S. aurita*. The Pauly (1984) method was used to estimate total mortality by using FISAT II (Gayanillo et al., 2005). The exploitation rate was estimated by the formula suggested by Gulland (1971) through the following relation: E = F/Z. Where: E = exploitation ratio, i.e., the fraction of deaths caused by fishing. F = fishing mortality coefficient. Z = total mortality coefficient. This is based on the assumption that a stock is optimally exploited at E = 0.5 when F equals M (Gulland, 1971) - E<0.5 underexploited stock, and E>0.5 overexploited stock.

RESULTS AND DISCUSSION

In this study, 894 individuals are treated, 546 females and 348 males.

The length frequency distribution of *S. aurita* by length and by sex, established monthly for the period from 2008 to 2009, is shown in figure 3. The total length of the samples during the sampling period ranged between 10.7 cm and 32.5 cm. The average length of the females (20.58 cm) is higher than that of the males (19.06 cm). The difference between the average sizes of females and males is significant ($\varepsilon > 1.96$). The minimum lengths are 10.7 cm for the females and 12.2 cm for the males, whereas the maximums are 32.5 cm for the females and 32 for the males. The length-frequency distribution throughout the study period shows a preponderant total length of 15 and 16 cm over others (Fig. 2). The length-weight relationship of *S. aurita* indicated a positive allometry for female and males and was found out to be WT=0.005 LT 3.08 for fe-

male and WT=0.005 LT 3.12 for males (Fig. 3). The analysis by sex showed a significant difference in the b coefficient. The degree of association between the two variables length and weight, is expressed by a correlation coefficient (r). The correlation coefficient is higher when its value is close to +1. The coefficient is estimated in this study at 0.97 for female and 0.98 for males. The computed annual mortality rates Z, M and F were 2.41, 0.79 and 1.62, respectively. The rate of exploitation (E) was estimated as 0.67, which indicate overfishing during the period of study and that the stock of *S. aurita* is heavily exploited. The maximum size (Lmax) attained by *S. aurita* in Oran waters is 32.5 cm while the smallest specimen measure 10.7 cm. Similar results were found by Bosiljka & Gorenka (2012) on the same species. The maximum size attained by an animal occurs when it grows to 95% of its asymptotic length according to the relationship Lmax = 0.95 L∞ (Moses, 1990). The length distribution of round sardinella indicates that the females were more present in longer length classes, especially in total length classes over than 26 cm; males were more frequent in the smaller ones. Such differences could be explained by lower mortality and higher growth in females than in males (Bosiljka & Gorenka, 2012). The von Bertalanffy model has been shown to have a better fit than other growth equations. In general, growth is affected by a variety of factors, such as food quantity and quality, and temperature

The results showed that the asymptotic length (L∞) of female (34.21 cm) and of male (33.68 cm) appeared to be higher than estimates reported by Chavance et al. (1985), on *S. aurita* caught in Oran bay. Nevertheless, the results are similar to those found by Bebars (1981), Bouaziz et al. (2001) and Dahel et al. (2016) (Table 1). The difference in a symptotique length can also be attributed to the fishing season, geographical reach of fishing activities and subsequently the dominating fish length during each fishing season, noise pollution from outboard motors and industrial activities, fishing pressure and environmental degradation (King, 1991). The estimate for growth coefficient (K) in this study is similar to observations for other populations (Chavance et al., 1985; Amrouche & Etsouri, 2006) (Table 1). Differences in growth patterns may be the result of differences in genetic structure and/or differences in temperature, density of food and diseases (Pauly 1994, Wootton 1998). Rohit et al. (2012) reported, the difference in growth rate can be attributed to several reasons including prevailing eco-biological conditions of the habitat from time to time. The exponent b of the length-weight relationship of the analyzed specimens showed positive allometric growth (b=3.08>3 for female and b=3.12 >3 for males) and indicates that the weight grows slightly faster than the size of the fish. The same values were found for the sardinella of the central region of the Algerian coast (Bouaziz et al., 1998), on the sardinella of the Tunis coasts (Kartas, 1981) and also in the Aegean Sea (Tsikliras et al., 2005). Claro & García-Arteaga (1994) indicate that this species also showed positive allometric growth. Once the growth parameters were known, it was possible to estimate the instantaneous total mortality. In the

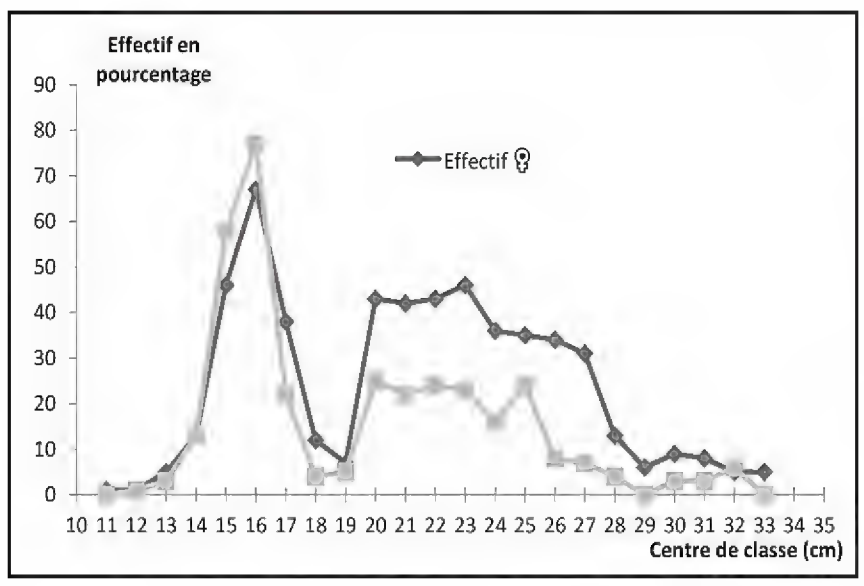

Figure 2. Length-frequency distribution by sex of *Sardinella aurita*.

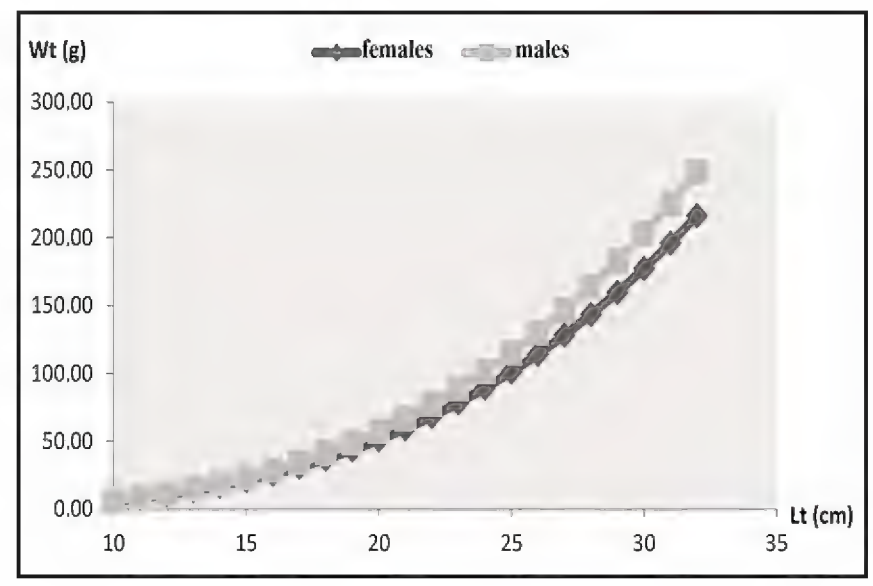

Figure 3. The length-weight relationship of *Sardinella aurita*.

Mediterranean Sea	Sex	L∞	K	T_0	Authors
Egypte	sexes combined	33.11	0.19	-1.34	Bebars (1981)
Algeria	sexes combined	34.96	0.23	-0.707	Bouaziz (2001)
Algeria	sexes combined	26.77	0.45	0	Amrouche & Etsouri (2006)
Oran western coasts	Females Males	25.5 22.9	0.52 0.64	——	Chavance et al. (1985)
Algeria eastern coasts	Females Males	32.26 27.3	0.13 0.18	 -1.9	Dahel et al. (2016)
Oran	**Female** **Male**	**34.21** **33.68**	**0.47** **0.39**	**-0.34** **-0.41**	**Present study**

Table 1. Comparison of growth parameters obtained from previous studies for *Sardinella aurita*.

Authors	Study area	Z (an^{1})
Bebars, 1981	Egypte	0.993
Chavance et al., 1985	Oran mâles	1.787
Bouaziz, 2007	Alger	2.51
Amrouche & Etsouri, 2006	Région centre de la côte algérienne	2.4
Sennai, 2003	Ghazaouet	0.96
Present study	Oran	2.41

Table 2. Comparison of total mortality coefficient obtained from previous studies for *Sardinella aurita*.

present investigation, suggested by length frequency distribution, the total mortality (Z), was estimated as 2.41 yr-1. It is defined as the total loss by natural and fishing death of individuals (Table 2).

In this study we observed that the mortality of *S. aurita* is affected more by exploitation of stock. Similar results were found on *S. aurita* of Algiers waters by Bouaziz (2007) and Amrouche & Etsouri (2006).

According to this study, the fishing mortality 1.62 yr-1was significantly higher than natural mortality 0.79 yr-1. The value of the natural mortality (M) obtained is very similar to the results of other authors such as Chavance et al. (1985) and Amrouche & Etsouri (2006). The natural mortality of *S. aurita* can be attributed to environmental stress due to the anthropic activities or to the Fish predation (tuna and other large pelagic fishes) (King, 1984). According to Amrouche & Etsouri (2006), the difference between the natural mortality coefficients can be explained by the climatic conditions of the environment, the season of study, the pollution and the predation. Belveze (1984) describes sardines and sardinella as an unstable and unpredictable species with high natural mortality. In this study, instead, the major cause of mortality of this species is due to fishing activity.

Fishing generally affects the age and length structure of fisheries stocks and fishing induced declines in the length structure (Stergiou, 2002). The present exploitation rate (E = 0.67) is higher than the optimum level (E = 0.5). This indicates the high vulnerability of the species to the fishing gear.

CONCLUSIONS

For a better management of the fishery, some recommendations should be carried out such as: (i) a thorough study of species-gear interactions (Panifili et al., 2002); (ii) conduct scientific fisheries to determine recruitment and selection sizes. In order to ensure sustainable exploitation of *S. aurita* stock, fishing effort should be regulated along with increase in mesh size. Restricting fishing outside the spawning season is considered necessary for sustainable exploitation of these stocks.

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Biodiversity Journal, 2019, 10 (4): 359–364 https://doi.org/10.31396/Biodiv.Jour.2019.10.4.359.364

Jujubinus silbogomerus n. sp. (Gastropoda Trochidae) from the Canary Islands, Atlantic Ocean

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ABSTRACT

Based on shell characters, a new species of the gastropod family Trochidae, *Jujubinus silbogomerus* n. sp., is described from the Atlantic Ocean. The new taxon is known from the type locality only, the Canary Islands. It was compared with the most closely related species of this geographical area as well as with the Atlantic and Mediterranean *Jujubinus* Monterosato, 1884 showing marked sculpture. The peculiar morphology of the teleoconch microsculpture and sculpture of this new taxon allows an easy identification.

KEY WORDS

Recent; Trochidae; *Jujubinus silbogomerus*; new species; Atlantic Ocean.

Received 19.10.2019; accepted 28.10.2019; published online 11.12.2019

INTRODUCTION

The genus *Jujubinus* Monterosato, 1884, currently under review (manuscript in preparation), actually is represented in the eastern European Atlantic Ocean and the Mediterranean Sea by 26 nominal species according to MOLLUSCABASE (http://www.molluscabase.org; last access October 2019). The shell of the genus *Jujubinus* typically displays a slender trochiform shape, with a marked sculpture consisting of 4 to 8 spirals often threads beaded and variable in size, with an evident basal cord, and with tiny prosocline lamellae within thread interspaces (Monterosato, 1884). The largest percentage of *Jujubinus* species are living in the intertidal zone down to about 80 meters, few can occur on the bathyal bottoms and are constantly associated with photophilic algal vegetation and/or marine phanerogames (Mariottini et al., 2013; Rolàn & Swinnen, 2013). In the Canary Islands, the genus *Jujubinus* has been studied in the past with the description of several species (Rolàn & Swinnen, 2009; Rolàn & Swinnen, 2013), which are mainly endemic of this geographical area. Living specimens of a new species, here described as *J. silbogomerus* n. sp., were sampled by SCUBA diving from the rocky bottoms of the island of Gomera, Canary Islands, off the coast of San Sebastian.

ACRONYMS. The materials used for this study are deposited in the following private and Museum collections: CS-PM: Carlo Smriglio-Paolo Mariottini, Rome, Italy; FS: Frank Swinnen, Lommel, Belgium; MCZR: Museo Civico di Zoologia di Roma, Rome, Italy; MNHN: Muséum National d'Histoire Naturelle, Paris, France; RBINS: Institus Royal des Sciences Naturelles de Belgique, Brussels, Bel-

gium. Other acronyms used in the text: H: Height; LIME: Interdepartmental Laboratory of Electron Microscopy; SEM: Scanning Electron Microscopy; shell (sh); W: Width.

MATERIALS AND METHODS

Twenty-one specimens (including juveniles, subadults and adults stages) of *J. silbogomerus* n. sp. were collected while SCUBA diving at a depth of 18 m in La Gomera Island (Playa la Cueva), Canary Islands, Spain. Sampling was consisting mainly of empty shells (shs), few individuals were collected alive. Scanning Electron Microscopy (SEM) photographs were taken at the Interdepartmental Laboratory of Electron Microscopy (LIME, Università "Roma Tre", Rome, Italy), using a Philips XL30. Current systematic here adopted is based on World Register of Marine Species (WoRMS, http://www.marinespecies.org/; last access October 2019). The *Jujubinus* species stored in the Monterosato's collection at the Museo Civico di Zoologia di Roma (MCZR) have been investigated for comparison with *J. silbogomerus* n. sp. In particular, shells of *Jujubinus tumidulus*: 1 sh labeled, "Trochus tumidulus Aradas Pliocen. Pleistocenico"; 2 shs labeled, "Jujubinus tumidulus (Aradas) Palermo", ex Bellini collection, Sicily, Italy; 123 shs labeled, "P", Palermo, Sicily, Italy, MCZR-M-11713, MTS; 13 shs labeled, "S. vito", San Vito Lo Capo, Trapani, Sicily, MCZR-M-E12/4, MTS; Linosa Island, Punta Calcarello, 36 m, Sicily, Italy, 11 shs, CS-PM; Linosa Island, 3 Ceppi, 28 m, Sicily, Italy, 50 shs, CS-PM; Maerettimo Island, 45 m, Sicily, Italy, 5 shs, CS-PM; Scilla, Strait of Messina, 42 m, Italy, 40 shs, CS-PM.

Shells of *Jujubinus montagui*: Shetland Islands, United Kingdom, 11 shs labeled, "Shetland M'Andr." (ex Mc Andrew collection), MCZR-M-E12/14, MTS; Aberdeen, United Kingdom, 5 shs labeled, "Trochus montacuti nt Aberdeen" (synonym), MCZR-M-E12, MTS; St. Malò, France, 9 shs, MCZR-M-11756, MTS; 1 sh labeled, "Jujubinus turgidulus Brocchi", MCZR-M-11713, MTS; Bastia, France, 5 shs, labeled "Trochus montacuti" ex Caziot collection, MCZR-M-11715, MTS; Capri, Italy, 2 shs, MCZR-M-11716, MTS; Sine Loco, "Coll Weink.", 1 sh, MCZR-M-11713, MTS; Anzio, 50 m, Latium, Italy, 120 shs, CS-PM; Fiumicino, fishing boats, 160 m, Latium, Italy, 4 shs, CS-PM; Caprera, Punta Crucitta, 78 m, Sardinia, Italy, 1 sh, CS-PM; San Vito, Trapani, Sicily, Italy, 7 shs, MCZR-M-11761, MTS; Sfax, 60 miles to the east, 100 m, Tunisia, 7 shs, CS-PM.

RESULTS

Systematics

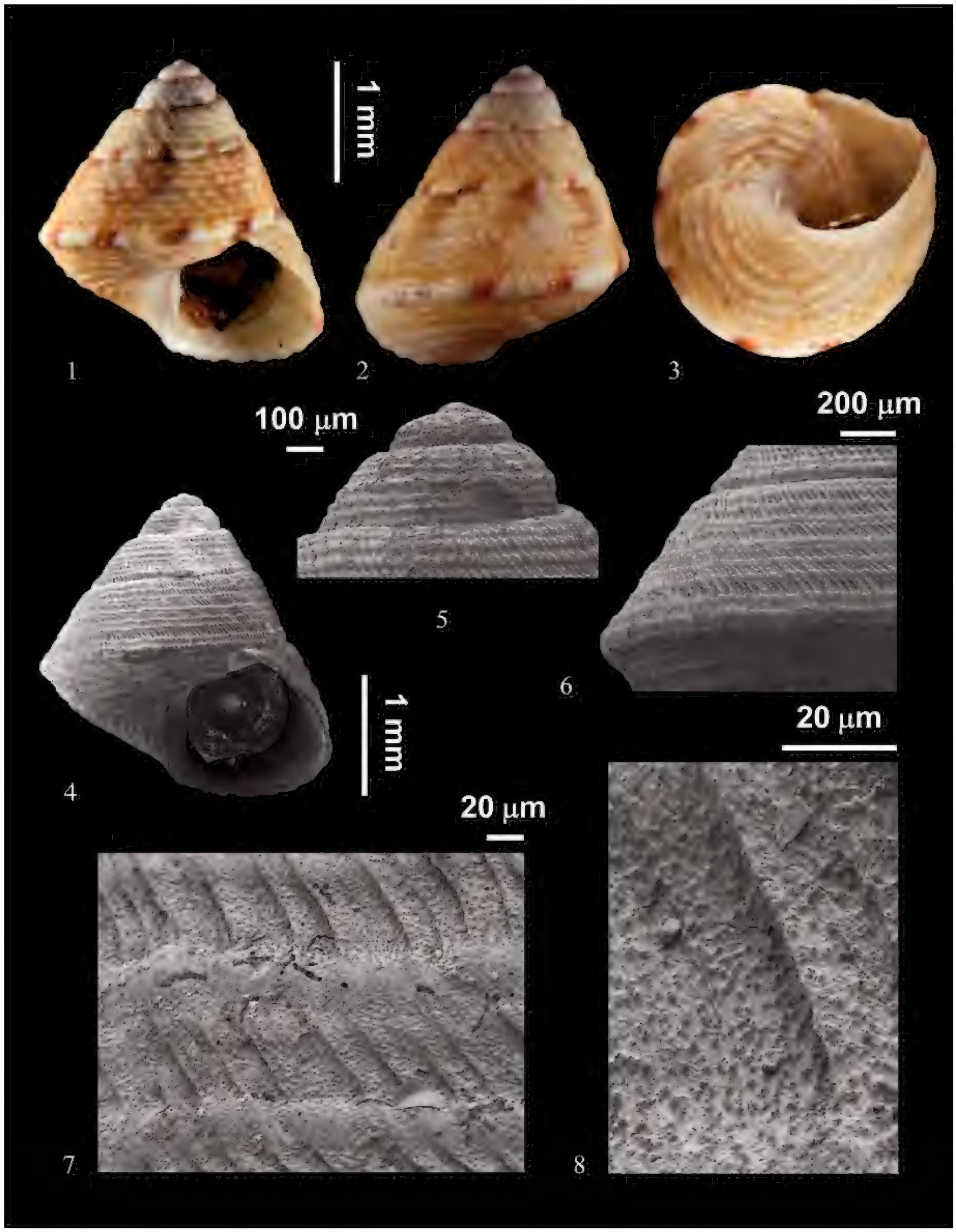
Classis GASTROPODA Cuvier, 1795
Subclassis VETIGASTROPODA Salvini-Plawen, 1980
Ordo TROCHIDA Rafinesque, 1815
Superfamilia TROCHOIDEA Rafinesque, 1815
Familia TROCHIDAE Rafinesque, 1815
Subfamilia TROCHINAE Rafinesque, 1815
Genus *Jujubinus* Monterosato, 1884
Type species: *Trochus matoni* Payraudeau, 1826 (by subsequent designation)

Jujubinus silbogomerus n. sp. (Figs. 1-17, 24-26)

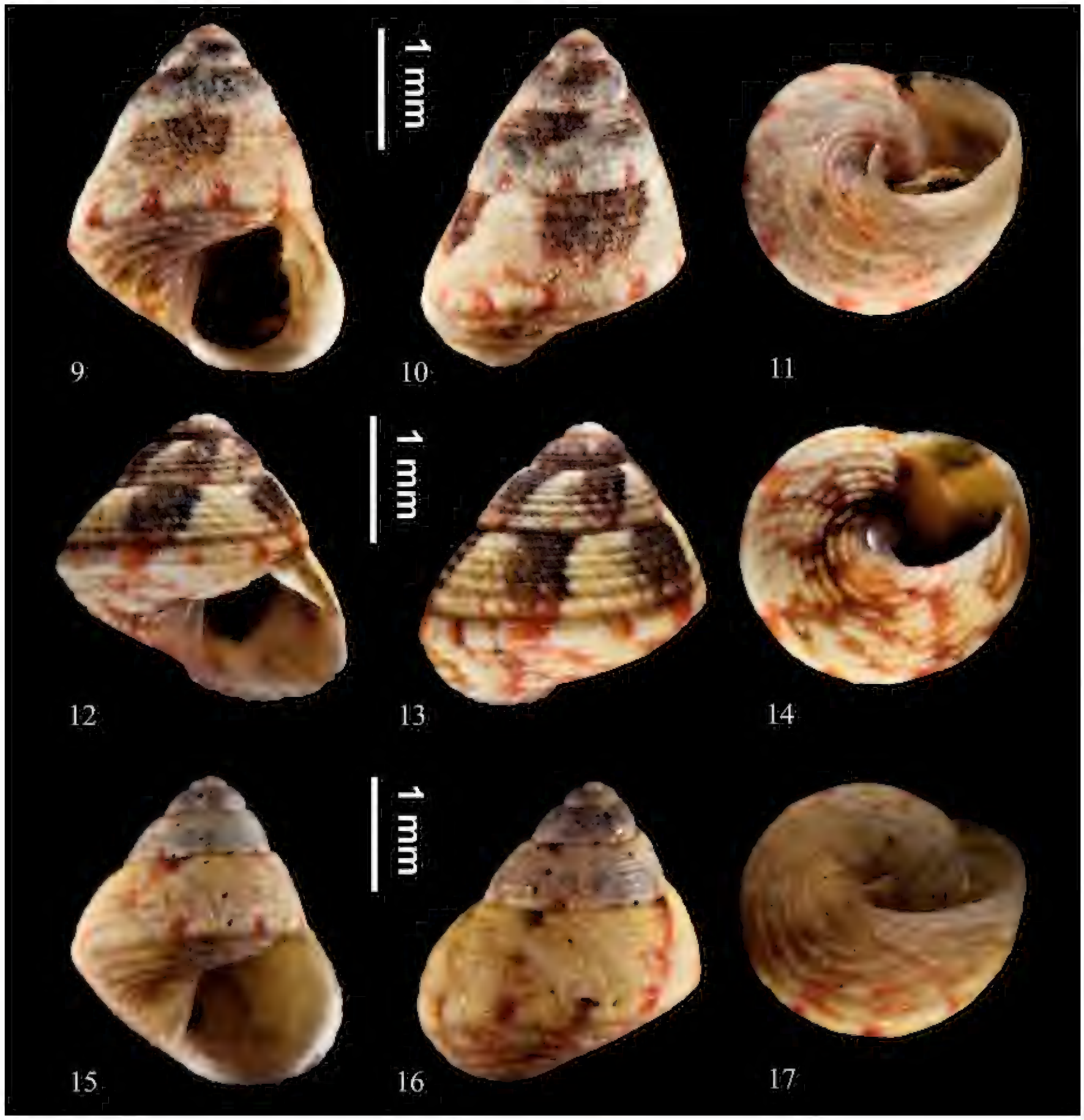
DIAGNOSIS. Small and little turriculate shell, with sculpture of incised spiral lines and strong prosocline lamellae between and over spiral cords, micropustules within interspaces.

EXAMINED MATERIAL. The holotype, MT.3820 / I.G. 34082, RBINS, and the paratype "A", MNHN-IM-2014-7994, the paratype "B, M-S" CS-PM coll.; the paratype "C-L", FS coll., all from type locality: La Gomera Island (off Playa de la Cueva, San Sebastian, 18 m deep, 28°06'N 17°06'W), Canary Islands, Spain, Atlantic Ocean; and three very young shells without soft parts, FS coll.

DESCRIPTION OF HOLOTYPE. Shell conical, of relatively small size for the genus, height (H) 2.8 mm, width (W) 2.8 mm, slightly shiny. Protoconch smooth of about 1.5 whorls. Teleoconch of 4.5 slightly convex whorls. Sculpture of 6 abapical spiral cords, regularly spaced and of about the same strength, with a strongly carved basal cord. Other basal cords narrow and well engraved, composed of two close threads, with very evident and strong lamellae in the interspace. First two whorls of the teleoconch showing a step on the shoulder and the spiral peripheral cord to the suture very highlighted

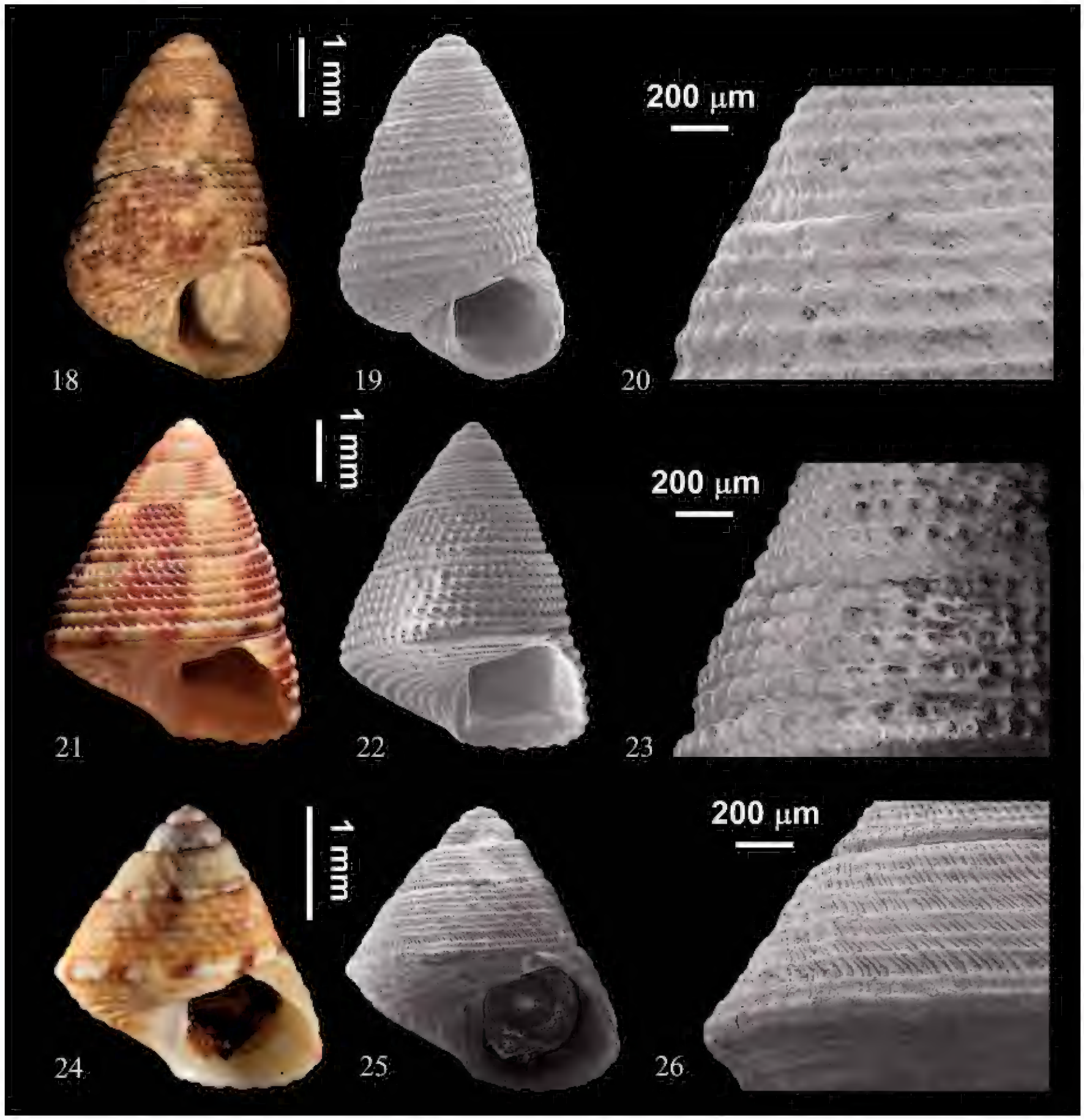


Figures 1–8. *Jujubinus silbogomerus* n. sp., holotype, La Gomera Island, Canary Islands, Spain, Atlantic Ocean. Figs. 1–3: shell, 2.8 (H) X 2.8 mm (W), frontal, dorsal and basal view, MT.3820 / I.G. 34082, RBINS. Fig. 4: idem, SEM uncoated, frontal view. Fig. 5: idem, SEM detail of the protoconch and of the first teleoconch whorls. Fig. 6: idem, SEM, detail of the shell whorl. Fig. 7: idem, SEM detail of microsculpture. Fig. 8: idem, SEM, detail of the surface covered by micropustule.



Figures 9–11. *Jujubinus silbogomerus* n. sp., Paratype "A", 3.0 (H) X 2.7 mm (W). MNHN-IM-2014-7994, La Gomera Island, Canary Islands, Spain, Atlantic Ocean; optical frontal, dorsal and basal views. Figures 12-14. *Jujubinus silbogomerus* n. sp., Paratype "B", 2.4 (H) X 2.5 mm (W), CS-PM, La Gomera Island, Canary Islands, Spain, Atlantic Ocean, optical of frontal, dorsal and basal views. Figures 15–17. *Jujubinus silbogomerus* n. sp., Paratype "C", 2.8 (H) X 2.5 mm (W), FS, La Gomera Island, Canary Islands, Spain, Atlantic Ocean, optical of frontal, dorsal and basal views.

from the ripple produced from the dense axial lamellae; last teleoconch whorls with a less pronounced basal cord. Suture incised. Surface of the teleoconch completely covered by very visible prosocline lamellae, arranged almost regularly, which end above the spiral cords, giving to the shell a crenulated appearance. Base convex, umbilicus closed and covered with a white callus. Aperture quadrangular, with the columellar callus positioned in the middle portion and internally whitish nacreous. Colour of protoconch whitish, teleoconch yellowish-creamy, with brownish quadrangular spots and basal cord coloured by short reddish - brownish spots. Basal disc yellowish interrupted by broad



Figures 18–20. *Jujubinus tumidulus,* Palermo, Italy, 4.8 (H) X 2.5 (W) mm, frontal view, MCZR-M-11713, MTS (Fig. 18); SEM frontal view, uncoated (Fig. 19); SEM detail of the shell (Fig. 20). Figures 21–23. *Jujubinus montagui*, 4.5 (H) X 3.6 mm (W), frontal view, CS-PM (Fig. 21); SEM frontal view, uncoated (Fig. 22); SEM detail of the shell (Fig. 23). Figures 24–26. *Jujubinus silbogomerus* n. sp., holotype, La Gomera, Canary Islands, 2.8 (H) X 2.8 mm (W), frontal view, MT.3820 / I.G. 34082, RBINS (Fig. 24); SEM frontal view, uncoated (Fig. 25); SEM detail of the shell (Fig. 26).

reddish-brownish lines decorated with 6-7 circular cords. Multispiral operculum.

VARIABILITY. Shell H ranging from 2.8 to 3.0 mm and W from 2.5 to 2.8 mm.

ETYMOLOGY. The species is named after the Silbo Gomero (whistle of La Gomera), the ancient whistle language which was used in the past by shepherds of La Gomera Island to communicate over great distances, now included in the oral and intangible heritage of humanity protected by UNESCO (United Nations Educational Scientific and Cultural Organization), (https://unesdoc.unesco.org, last access 10. 02. 2019).

DISTRIBUTION AND BIOLOGY. Currently only known from La Gomera Island, Atlantic Ocean, type locality.

This species seems to prefer habitat with poor vegetation and bottoms with hard substrate from which come the majority of living specimens object of the present work.

DISCUSSION AND CONCLUSIONS

Jujubinus silbogomerus n. sp. has very peculiar shell morphology, consisting in a strong sculpture enriched by the typical interspaced prosocline lamellae, which are dense and producing a rough looking shell surface. This peculiar sculpture was never described in the species attributed to the genus *Jujubinus* and it is comparable only with that of *J. montagui* (Wood, 1828) known for Mediterranean, European Atlantic, Morocco and the Canary Islands (Hernàndez et al., 2011) and Madeira (Segers et al., 2009), and that of *J. tumidulus* (Aradas, 1846), which seems to be geographically present only in the Mediterranean Sea, mostly localized in the southern Tyrrhenian Sea (Scaperrotta et al., 2009). *Jujubinus montagui* also shows a strong and exalted sculpture with tuberculate spiral cords and small interspaces containing short, robust and spaced prosocline lamellae, but not as dense as the new taxon. Furthermore, *J. montagui* has a larger size and different general shape and colour, while *J. silbogomerus* n. sp. is provided of small spiral cords sculpted but inconspicuous with interspaces totally occupied by prosocline lamellae closely spaced with micropustules, absent in *J. montagui*, that cover the entire surface. *Jujubinus tumidulus* is also provided with an exalted sculpture, but the spiral cords are highly tuberculated with spaced lamellae in the interspaces but without micropustules. This species has a very turreted shape, while *J. silbogomerus* n. sp. is rather compressed in outline, which shows also a different colour pattern. Currently, in both Atlantic Ocean and Mediterranean Sea only *J. silbogomerus* n. sp. presents this morphology and unique peculiar teleoconch sculpture, allowing its identification easily and unambiguously.

ACKNOWLEDGEMENTS

We thank very much Andrea Di Giulio (Dipartimento di Scienze, Università Roma Tre, Rome, Italy) for the SEM photographs carried out at the LIME (Laboratorio Interdipartimentale di Microscopia Elettronica, University Roma Tre, Rome, Italy). We would like to express our gratitude to Massimo Appolloni (Museo Civico di Zoologia, Rome, Italy) for the examination of the *Jujubinus* material kept in the Monterosato collection.

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Biodiversity Journal, 2019, 10 (4): 365–368 https://doi.org/10.31396/Biodiv.Jour.2019.10.4.365.368

Forty-seven years later: the blue crab *Callinectes sapidus* Rathbun, 1896 (Crustacea Decapoda Portunidae) reappears in the Strait of Messina (Sicily, Italy)

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ABSTRACT The second record of the invasive blue crab *Callinectes sapidus* Rathbun, 1896 (Crustacea Decapoda Portunidae) from the Strait of Messina has been reported, forty-seven years after the first record in this area. The specimen, a large-sized adult female, was collected in the Capo Peloro Lagoon, a natural reserve whose brackish waters represent a highly favorable environment for this euryhaline opportunistic species. The actual possibility that *C. sapidus* may settle in the Lagoon should be carefully evaluated, and a mitigation strategy, involving selective removal of pioneer specimens, timely envisaged.

KEY WORDS Crustaceans; Invasive species; Mediterranean; Brackish environments; Natural Reserves.

Received 16.10.2019; accepted 30.10.2019; published online 11.12.2019

INTRODUCTION

The American blue crab *Callinectes sapidus* Rathbun, 1896 (Crustacea Decapoda Portunidae), whose areal ranged from Canada to Argentina along the whole Atlantic western coasts (Nehring, 2011), in the early 20th century spread eastward, reaching the European coasts and, in order, North Sea, Mediterranean Sea, Baltic Sea, Black Sea and probably the Sea of Azov (Nehering, 2011, and references cited therein). All records, nevertheless, for over half a century were almost scattered, suggesting multiple independent introductions, with ballast water as the major introduction vector, without any success over time. The actual settlement of *C. sapidus*, which is now recognized as one of the worst invasive species in the Mediterranean Sea (Zenetos et al., 2005), has been thus dated back to the beginning of the 21st century (Galil et al., 2008), with first evidences of a still ongoing colonizing wave (Mancinelli et al., 2013; Karachle, 2013; Garcia et al., 2018; Piras et al., 2019). In Sicily, records of *C. sapidus* have been reported from the Strait of Messina (Cavaliere & Berdar, 1975), eastern coasts of Sicily (Franceschini et al., 1993) and Strait of Sicily (Insacco & Zava, 2017), but evidences on established populations in Sicilian waters are lacking.

MATERIAL AND METHODS

Brackish benthic communities in the Strait of Messina area have been monthly monitored since 2008, in the framework of the program: "Settlement dynamics and colonization of allochthonous assemblages in the Capo Peloro Lagoon". The lagoon is a Natural Reserve consisting of two differently fea-

tured basins, Lake Faro and Lake Ganzirri, connected to each other and to the sea by canals (Fig. 1). The Lake Ganzirri (38°15'38"N; 15°37'2"E) is a microtidal coastal pond, covering a 34 ha area and reaching 7 m maximum depth. It is connected to the sea by the "Due Torri" canal, in the north, while in the south a minor canal, "Catuso", allows faint water exchange.

The monitoring, carried out by snorkelling and SCUBA diving from 0 m to 3 m depth in the lake Ganzirri and related canals, provided specimen collections and photo documentation, now available at the Benthic Ecology Laboratory, Messina University.

RESULTS

A blue crab specimen was found on 22 May 2019, during the normal monitoring activities carried out in the Peloro Lagoon. In particular, a not ovigerous female was photographed in vivo (Fig. 2) in the inlet of the "Due Torri" canal, on gravelly sand, 0.1 m depth, during change of tide and full moon.

The crab specimen, handily collected, was identified in laboratory as *C. sapidus*, according to Williams (1974). It measured 74.8 mm in carapace length, CL, and 184.0 mm in carapace width, CW (Width / Length ratio = 2.46), corresponding to the largest size of adult females (Williams, 1974), as also observed in established Mediterranean populations (Türeli et al., 2016).

This basic measures and other morphometric parameters are synthesized in Table 1.

The living specimen dorsally displayed a grayish, bluish to brownish green color, with orange fingers on chelae tipped with purple, and under parts off-white with tints of yellow and pink, as characteristic of adult females. The specimen has been deposited as dried sample in the scientific collection of the Benthic Ecology Laboratory (ChiBioFarAm Department, Messina University), with reference code BEL135CRGANZ3Ce.

DISCUSSION AND CONCLUSIONS

The actual occurrence of *C. sapidus* in Sicilian waters, so far attested by occasional records, is questionable. Insacco & Zava (2017), reporting the "First record of the Blue Crab *Callinectes sapidus* in Sicily", did not give credit to Franceschini et al. (1993) which cited *C. sapidus* for the Ionian coasts of Sicily, neither to Cavaliere & Berdar (1975) which reported two female specimens from the Strait of Messina. Formerly, Galil et al. (2011), have considered unconfirmed the reports from the Ionian coasts, because lacking of details, but they credited the records from Messina, whose photos and synthetic description allow an unambiguous attribution

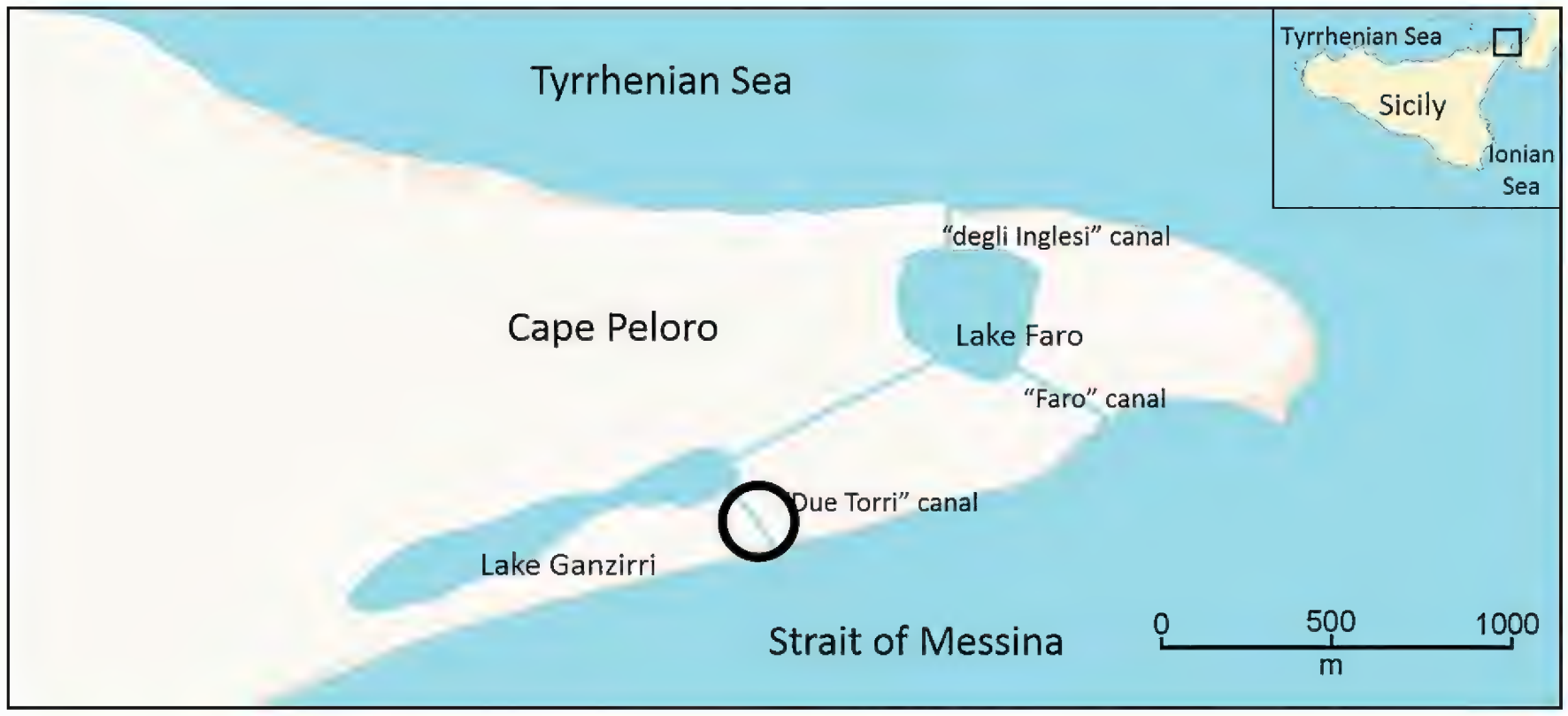

Figure 1. The Cape Peloro Lagoon. The circle indicates the sampling site, inside the "Due Torri" canal.

Fig. 2. In vivo photo of the described specimen, the American blue crab *Callinectes sapidus*.

Carapace Length CL	74.8 mm
Carapace Width CW	184.0 mm
Lateral Spine Length LSL	25.7 mm
Front Margin FM	28.4 mm
Hand Length HL	73.4 mm
Lateral Posterior Margin LPM	78.0 mm
Lateral Anterior Margin LAM	75.4 mm
Orbit Width OW	19.2 mm
Abdomen Posterior Width APW	57.8 mm
Abdomen Width AW	57.2 mm
Abdomen Length AL	54.5 mm
Total Abdomen Length TAL	72.3 mm

Table 1. Morphometric data concerning the *C. sapidus* specimen.

to *C. sapidus*. Such specimens, initially held in the local aquarium collection, are now awaiting inventory at the "Museo della Fauna", Messina University. The present record is thus the second one from the Strait of Messina, almost half a century after Cavaliere & Berdar (1975), and the third one ascertained from Sicilian waters, after Insacco & Zava (2017). None of these records may be considered a prove of *C. sapidus* settlement, but the possibility that C. sapidus may settle in Lake Ganzirri should be carefully evaluated. This highly invasive euryhaline species, in fact, might take advantage by the absence of a competitive native fauna (Bottari et al., 2005). Fishing, furthermore, is forbidden in the Peloro Lagoon Natural Reserve, so that the recent suggestion to mitigate the *C. sapidus* impact by promoting its value as a fishery resource (Mancinelli et al., 2017), is currently unthinkable.

A conservation strategy involving selective removal of pioneer specimens, in our advise, should be thus timely envisaged.

ACKNOWLEDGENENTS

This research was sponsored by the 'Città Metropolitana di Messina' the official management body

of the reserve (permission no. 557/VIII DIR, 12.03.2014). Facilities were provided by the mussel farm FARAU s.r.l.

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DECEMBER 2019, 10 (4): 369-566

www.biodiversityjournal.com

ISSN 2039-0394 (Print Edition)
ISSN 2039-0408 (Online Edition)

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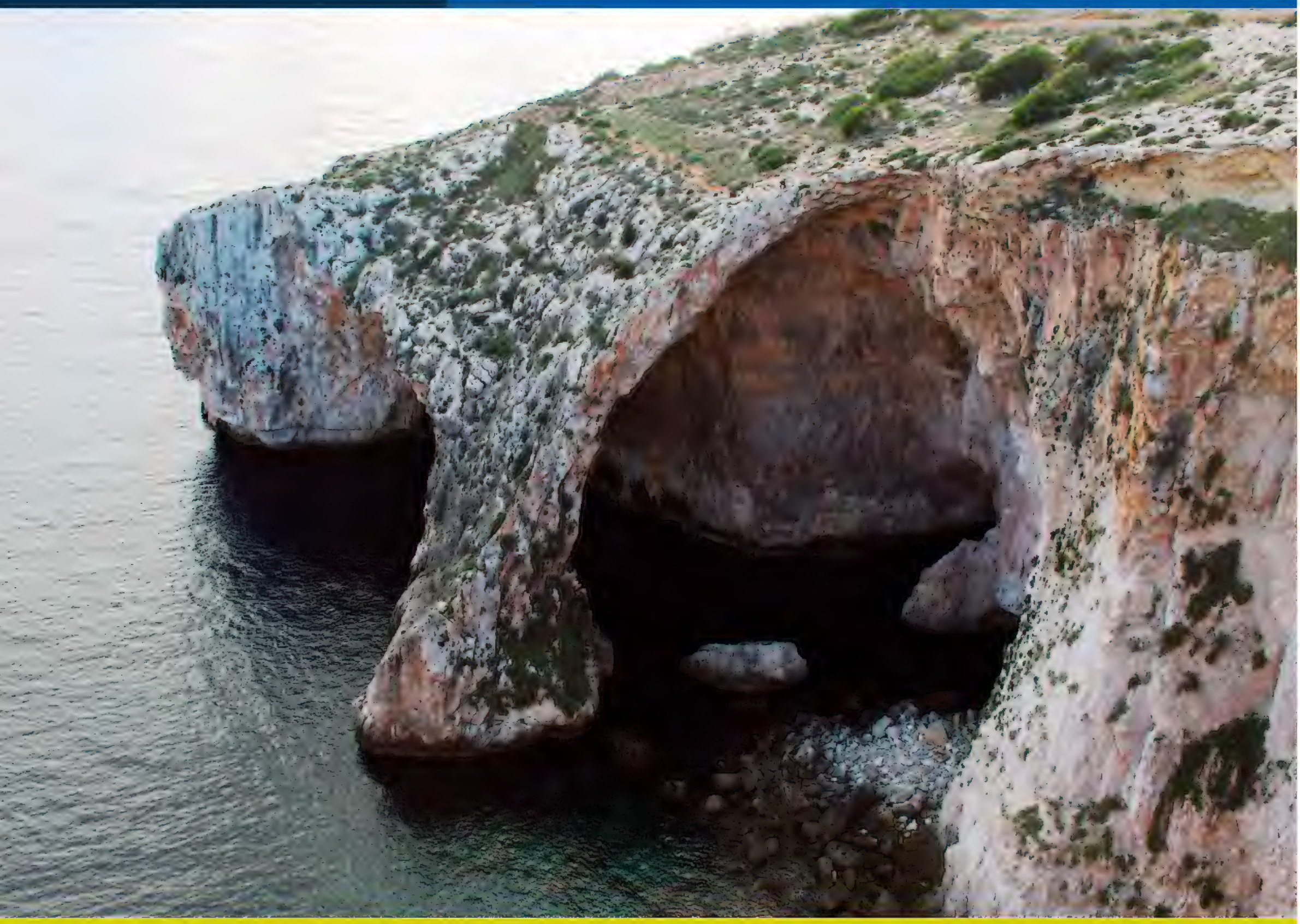
Biodiversity Journal

MONOGRAPH

PROCEEDINGS OF THE 4th INTERNATIONAL CONGRESS ON BIODIVERSITY "MAN, NATURAL HABITATS AND EURO-MEDITERRANEAN BIODIVERSITY

NOVEMBER 17th-19th, 2017 - MALTA



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Biodiversity Journal, 2019, 10 (4): 371–372 https://doi.org/10.31396/Biodiv.Jour.2019.10.4.371.372
MONOGRAPH

Introduction

Considerations on the 4th International Congress on Biodiversity "Man, Natural Habitats and Euro-Mediterranean Biodiversity"

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Received 06.12.2019; accepted 12.12.2019; published online 20.12.2019
Proceedings of the 4th International Congress on Biodiversity "Man, Natural Habitats and Euro-Mediterranean Biodiversity", November 17th-19th, 2017 - Malta

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There is no doubt that the history of naturalistic knowledge is as long as the history of man. The observation of nature has followed the progress of our species at least since our most distant ancestors tried to impress shapes and images on the rocky walls of what they observed around them, also trying to interpret the hidden meanings to understand their essence. And since then we have continued to do so.

In his "Metaphysics", Aristotle argued that man aspires to knowledge by its natural inclination. And perhaps it is no coincidence that "The Stagirita" in the study of nature ends up immersing itself at the peak of its maturity in the last years of its life (and its production on the subject is monumental): it is probably in the nature that he ultimately sought to find ever more extensive and in-depth answers to fundamental questions which we still place today as a refrain: who we are, where we come from, where we are going.

A naturalistic investigation at the bottom is nothing but a piece of a puzzle, the one through which we humans try to complete the framework without boundaries of knowledge. "*A painting without borders*": undoubtedly an oxymoron that nevertheless contains in itself the fascination of continuous discovery that accompanies the ability to marvel in discovering the new.

With a stupendous phrase, Albert Einstein suggests that "*The important thing is to never stop asking. Curiosity has its reason to exist. One can do nothing but be amazed when one contemplates the mysteries of eternity, of life, of wonderful structure of reality. It is enough if we try to understand only a little of this mystery every day. Never lose a sacred curiosity*". In these words is condensed the meaning , and I would like to say the mission, that every good researcher should set for himself as goal of his journey: this still in the spirit of that research giant that suggested that "*The joy of observing and understanding is the most beautiful gift of nature*".

Well, I am convinced that the cultural value of this volume must be sought in these considerations: it is a further contribution to the knowledge of nature, in particular of biodiversity, also in view of its protection in the path already traced by the now historic Rio Conference of 1992 which stressed out key objectives: the conservation of biodiversity, the sustainable use of biodiversity, a fair and equitable

distribution of the benefits deriving from the use of genetic resources, ultimately encouraging actions that can propitiate a sustainable future. We all see how this target has not yet been fully achieved, despite efforts to do so, on the contrary, there are very many indicators that tell us otherwise. But it is equally evident that the guard, the political, social, economic and cultural effort to reverse that trend cannot but be strengthened.

Studies and research such as those that the volume contains go in that direction. They are the essential, initial step to protect biodiversity: first of all get to know it. "*One cannot love what one does not know*", argued Fyodor Dostoyevsky in his famous novel "Besy". Are words that echo in the sentence of Giulia Maria Crespi, founder of the FAI, "*You protect what you love and love what you know*". Therefore the protection of biodiversity starts from its knowledge and Biodiversity Journal works in this sense.

But this volume also contains a further specific added value that is linked to its long-term programmatic design. It includes research that has been exhibited during the 4th International Congress on Biodiversity which, with the subtitle "Man, Natural Habitat and Euro Mediterranean Biodiversity" was hosted by the University of Malta. So it was the fourth in a series of conferences dedicated to biodiversity: the first in Palermo in December 2012, the second in Cefalù in 2014, the third in 2016 in Noto, while a fifth was celebrated in Sofia in 2019 and already is forecasted the celebration of a sixth congress. It is therefore a scientific project characterized by a cultural continuity that aspires to continue. Moreover two peculiar aspects are added to the objective of communicating and spreading biodiversity surveys: the first is the conjunction of the pure research with the biodiversity-human relations and interactions, the second is the accentuation of the attention on Euro-Mediterranean biodiversity.

Furthermore, this series of scientific appointments have as goal to represent moments of periodical meeting and comparison between researchers who also intend to build a scientific community in which to graft and enhance human relationships strengthening them by means of the periodicity of the exchanges also aiming to the enlargement of the collaborations. An attempt therefore to create an ever wider cenacle where information, experiences, proposals, solidarity and friendship are exchanged.

It therefore seems to us of particular value and importance the will to share a path in which alongside a vision of biodiversity integrated with the environmental problems, and not only that, a chorus of components and subjects emerges that contribute to the realization of the common goal. To date, the actors of this project, alongside Biodiversity Journal which first launched it, were the Ente Fauna Siciliana (Naturalistic Association of Noto, Siracusa), the University of Catania, the University of Malta, the Sofia University, Chloe (Naturalistic Association of Strongoli, Catanzaro), thanks to the support of scientific committees made up of qualified and authoritative scholars.

Finally, a thank you goes to all the participants of the 4th congress of the series for the richness of their contributions, as can be seen reading not only this volume but also the others that are part of our scientific itinerary. Thanks also to all representing the numerous authoritative institutions that attend this ideal congressional-cultural relay race that we will try to carry on.

Biodiversity Journal, 2019, 10 (4): 373–382 https://doi.org/10.31396/Biodiv.Jour.2019.10.4.373.382

MONOGRAPH

Terrestrial mammals of the satellite islands of Sardinia (Italy)

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ABSTRACT

The mammalian fauna of the satellite islands and islets of Sardinia (Italy) is still imperfectly known. Only few data are available for some of them, while several others are still almost regarded as terra incognita. Complete information on the extant non-volant terrestrial mammals is available only for Asinara, Tavolara, and Molara, whereas historical and present data are available on the mammals of San Pietro. Further information on the non-flying taxa occasionally appears in specialised literature. At present, 15 species occur in the circum-Sardinian archipelagos. Only 6 of them, however, have been reported from the largest island, San Pietro, whereas the smaller Asinara hots at least 13 species, excluding local domestic breed such as the dwarf donkey. Data on the distribution of chiropters have been provided by a few studies carried out over time. Of the 21 species of bats found in Sardinia, at least 11 were recorded from the small islands.

KEY WORDS

Non-volant terrestrial mammals; bats.

Received 13.06.2018; accepted 05.10.2018; published online 20.12.2019

Proceedings of the 4th International Congress on Biodiversity "Man, Natural Habitats and Euro-Mediterranean Biodiversity", November 17th-19th, 2017 - Malta

INTRODUCTION

The mammalian fauna of the satellite islands and islets of Sardinia (Italy) is still imperfectly known.

Only few data are available for some species, while several others are still almost regarded as "*terra incognita*". Complete information on the extant non-volant terrestrial mammals is available only for Asinara (Torre & Monbailliu, 1993; Cossu et al., 1994), Tavolara (Ranzi, 1971; Trainito, 2008), and Molara (Sposimo et al., 2012), whereas Zava et al. (1995) make available historical and present data on the mammals of San Pietro.

Further information on the non-flying taxa occasionally appears in specialised literature.

DISCUSSION

At present, 15 species occur in the circum-Sardinian archipelagos. Only 6 of them, however, have been reported from the largest island, San Pietro, whereas the smaller Asinara hots at least 13 species, excluding the local dwarf donkey domestic breed. The only species which appears to be more widespread is the black rat, *Rattus rattus* (Linnaeus, 1758), occurring almost everywhere, a part from the islets of Spargiotto, Barrettini, and Foradada (Capo Caccia) (Martin et al., 2000).

The brown rat, *Rattus norvegicus* (Berkenhout, 1769), has been instead reported only from Asinara (Sarà, 1998) and Tavolara (Capizzi & Santini, 1999e, 2002e; Trainito, 2008, 2011). Recent surveys, however, did not confirm the occurrence of

this species on the latter island (Paolo Sposimo, 2014 pers. com.). The garden dormouse, *Eliomys quercinus* (Linnaeus, 1766) (Fig. 1), the wood mouse, *Apodemus sylvaticus* (Linnaeus,1758), and the weasel, *Mustela nivalis* Linnaeus, 1766 (Fig. 2), are present on Asinara.

Another carnivore, the fox, *Vulpes vulpes* (Linnaeus, 1758), has been reported from Isola dei Cavoli where it was introduced after 1995 to prevent the damages produced by the rats, although its presence was defined by Scrugli & Cogoni (1995) "*as only occasionally verifiable*". Observations of this carnivore are also available for San Pietro (Zava et al., 1995). The western European hedgehog, *Erinaceus europaeus* Linnaeus, 1758, occurs on Asinara (Torre & Monbailliu, 1993; Cossu et al., 1994; Amori & Masseti, 1996), San Pietro (Zava et al., 1996), and Caprera (Cossu et al., 1994). The North African white-toothed shrew or Pantellerian shrew, *Crocidura pachyura* (Küster, 1835) inhabits Asinara (Amori & Masseti, 1996; Torre & Monbailliu, 1993), and Caprera (Amori & Masseti, 1996; Thibault et al., 1988), whereas the pygmy white-toothed shrew, *Suncus etruscus* (Savi, 1822) has been only certainly reported from Asinara (Torre & Monbailliu, 1993; Amori & Masseti, 1996; Contoli & Amori, 2008). On Tavolara, data on the presence of the latter species are only known from the first half of 1970s (Ranzi, 1971; Pratesi & Tassi, 1973). Together with the house mouse, *Mus musculus* Linnaeus, 1758, dispersed on Asinara (Torre & Monbailliu, 1993; Amori & Masseti, 1996; Capizzi & Santini, 1999c, 2002c), Tavolara (Baccetti et al., 2009; Sposimo et al., 2012; Ragionieri et al., 2013), Isola Piana, Isola dei Cavalli, Proratora, Reulino or Isolotto rosso (present work), the rabbit, *Oryctolagus cuniculus* (Linnaeus, 1758), is one of the most widespread species and occurs on Isola Piana (off Tavolara) (Trainito, 2008; Trainito & Navone, 2011), San Pietro (Cetti, 1774; Vallebona, 1974; Racheli, 1981; Zava et al., 1996), Caprera, Santa Maria, Razzoli, Spargiotto, Spargi, Mal di Ventre, Vacca, Toro, and Isola Rossa (Spagnesi, 1999b and 2002b). Pratesi & Tassi (1971, 1973) noted that the latter species was also reported from Tavolara, but it is no more present. According instead to Segala (1991) and Spagnesi (1999a and 2002a), the brown hare, *Lepus europaeus* Pallas, 1778, occurs on Asinara; Spagnesi (1999a, 2002a) reported the occurrence of this species from Maddalena and San Pietro, too.

The situation of the ungulates is rather different and only three species of this taxonomic group are presently known for the satellite islands of Sardinia. Ancient populations of wild goats, *Capra aegagrus* Erxleben, 1777, are traditionally reported from Asinara (Toschi, 1953; De Beaux, 1955; Couturier, 1959; Massoli Novelli, 2003; Masseti, 2008, 2009, 2014) (Fig. 3) and Tavolara (Cetti, 1774; Valery, 1837; McGrigor, 1866; Toschi, 1953; Couturier, 1959; Masseti, 2008, 2009, 2014). Nowadays, their occurrence on both islands appears seriously compromised, and no individual of the original population still remains on Tavolara, where domestic goats were introduced by man. Most of the human population of Tavolara was displaced in 1962 when a

Figure 1. The garden dormouse, *Eliomys quercinus* (Linnaeus, 1766), is among the non-volant mammals present on Asinara (photo by Roberto Meloni).

Figure 2. The weasel, *Mustela nivalis* Linnaeus, 1766, is the sole carnivore which occurs on Asinara (photo by Riccardo Romanelli).

NATO radiogoniometric station was constructed on the eastern half of the island. As a result of this, the animals escaped from their guardians' control giving origin to a new population, the descendants of which have survived up to the present. According to Ruiu & Trainito (1999), Tavolara represents the territory in which the Sardinian subadults of golden eagle, *Aquila chrysaetos* (Linnaeus, 1758), coming in particular from the hills of Gallura and Monte Nieddu, spend their first winter to practice hunting techniques: "*They mostly look for goats. First hair kids, to claw on the fly to avoid the furious defense of adult*" individuals of the new population of feral goats today available on the small island. According to Couturier (1959), the original wild goats of Tavolara were instead the same as the goats of the northern Tyrrhenian island of Montecristo. They were characterised by morphological patterns which fall within the phenotype of *Capra aegagrus dorcas* Reichenow, 1888, typical of the island of Youra, in the Northern Sporades archipelago (Western Aegean Sea, Greece) (Masseti, 2009). A few original wild goats of Tavolara were collected before their extinction in the wild, and were bred in a free-ranging state until recently in the Ogliastra bush, within the perimeter of the Gulf of Orosei and Gennargentu National Park, where they returned to the wild after their abandonment by the shepherds (Fig. 4). They survived in the new territories of distribution at least up to the end of the first decade of 2000s. It seems that these goats were brought from Tavolara over 120 years ago, in the second half of the 800s.

Mouflons, *Ovis orientalis* (Gmelin, 1774), are only present on Asinara (Torre & Monbailliu, 1993; Massoli Novelli, 2003), and Figarolo (Pratesi & Tassi, 1973; Ruiu, 1993; Bocchieri & Satta, 1999; Trainito, 2008; Trainito & Navone, 2011) (Fig. 5). The stock of Asinara was introduced from the Natural Reserve of Capo Figari and the islet of Figarolo in 1952. Wild boars, *Sus scrofa* Linnaeus, 1758, occur permanently only on five islands: Asinara (Torre & Monbailliu, 1993; Pedrotti & Toso, 1999, 2002; Massoli Novelli, 2003), Spargi (Racheli; Pedrotti & Toso, 1999, 2002), Caprera (Pedrotti & Toso, 1999, 2002), Maddalena (Pedrotti & Toso, 2002) and Sant'Antioco, but they have been also reported swimming from Asinara to the near Isola Piana (Bazzoni, 2013). Modern scientific investigation considers wild boars as quite

Figure 3. Trophy of an adult male captured on the small island of Asinara in 1897, and preserved in the collection of the Museum of Natural History of the University of Florence Zoological Section (MZUF no. coll. 11943) (photo by Saulo Bambi, courtesy Zoological Museum "La Specola" of the University of Florence).

competent swimmers, although they cannot survive a crossing of more than a few miles of open sea. In fact, they have been often reported swimming across the narrow marine straits separating the islets from the continental shores of the Mediterranean (Masseti, 2007, 2012). All the wild boars have been introduced on the satellite islands of Sardinia for hunting purposes in rather recent times. Regarding the ungulate domestic breeds, Sardinia has always been considered the stronghold of donkeys. Nowadays, however, these equids are no longer as widespread as they were in the past. In a still recent past, they demonstrated a substantial variation in size, ranging from normal dimensions to those typical of the Mediterranean dwarf varieties (cf. Masseti, 2012), like the donkeys that are still reared on the island of Asinara (see Pinna et al., 1990; Pinna et al., 1993; Cossu et al., 1994) (Fig. 6).

Data on the distribution of chiropters on the satellite islands of Sardinia have been provided by Zava & Violani (1992), Grafitti & Mucedda (1995), Zava et al. (1995), Fornasari et al. (1997), Mocci Demartis & Secci (1997), Skiba (2009), Winter et

al. (2015). Very recently, Mucedda et al. (2016) published an important overview of the bats of La Maddalena, Caprera, Santo Stefano, Spargi, Budelli, Santa Maria, Tavolara, Molara, Figarolo, Asinara, Isola Piana (off Asinara), San Pietro, Sant'Antioco, Serpentara and Isola dei Cavoli. Of the 21 species of bats found in Sardinia, at least 11 were recorded from the satellite islands. The species most widespread is the common pipistrelle, *Pipistrellus pipistrellus* (Schreber, 1774), which is present on 15 islands, followed by the European free-tailed bat, *Tadarida teniotis* (Rafinesque, 1814) (Fig. 7), on 12 islands. The Savi's pipistrelle, *Hypsugo savii* (Bonaparte, 1837), and the Kuhl's pipistrelle, Pipistrellus kuhlii (Kuhl, 1817) are instead reported from nine islands. The rarest species is the Daubenton's bat, *Myotis daubentonii* (Kuhl, 1817), which has been observed only on Asinara. No individual of the endemic Sardinian long-eared bat, *Plecotus sardus* Mucedda, Kiefer, Pidinchedda and Veith, 2001, has been recorded from any of the islets, being its distribution limited to a few wooded areas of central and central-eastern Sardinia. Mucedda et al. (2016) are also of the opinion that the lesser mouse-eared bat, *Myothis blythi* (Tomes, 1857), has been erroneously reported from San Pietro by Zava & Violani (1992), since the only two species of the genus *Myotis* occurring in Sardinia are the long-fingered bat, *M. capaccini* (Bonaparte, 1837) - reported by Capizzi & Santini (1999d, 2002d) from La Maddalena, and Mucedda et al. (2016) from San Pietro - and the Maghreb

Figure 4. Original wild goats of Tavolara, before their extinction in the wild, were bred in a free-ranging state until recently in the Ogliastra bush, Gulf of Orosei and Gennargentu NP.

Figure 5. Introduced populations of mouflons, *Ovis orientalis* (Gmelin, 1774), are present today on Asinara and Figarolo (photo by Marco Masseti).

Figure 6. A small race of donkey is still bred on the island of Asinara, off the northwestern coast of Sardinia (photo by Marco Masseti).

Figure 7. Portrait of an adult European free-tailed bat, *Tadarida teniotis* (Rafinesque, 1814) (photo by Bruno Zava).

island	species	references
Razzoli	*Oryctolagus cuniculus*[1]; *Rattus rattus*[2-3];	[1]Spagnesi, 1999b, 2002b; [2]Capizzi & Santini, 1999d, 2002d; [3]Martin *et al.*, 2000;
Budelli	*Pipistrellus pipistrellus*[1,3]; *Tadarida teniotis*[3]; *Rattus rattus*[2];	[1]Mucedda *et al.*, 2015; [2]Martin *et al.*, 2000; [3]Mucedda *et al.*, 2015;
Spargiotto	*Oryctolagus cuniculus*[1];	[1]Spagnesi, 1999b, 2002b;
Spargi	*Pipistrellus pipistrellus*[7,8]; *Pipistrellus pygmaeus*[8]; *Miniopterus schreibersi*[8]; *Hypsugo savii*[8]; *Tadarida teniotis*[8]; *Oryctolagus cuniculus*[1]; *Sus scrofa*[2,3,4]; *Rattus rattus*[5,6];	[1]Spagnesi, 1999b, 2002b; [2]Racheli; [3]Pedrotti & Toso, 1999; [4]Pedrotti & Tosso, 2002; [5]Capizzi & Santini, 1999d, 2002d; [6]Martin *et al.*, 2000; [7]Mucedda *et al.*, 2015; [8]Mucedda *et al.*, 2015
Santa Maria	*Oryctolagus cuniculus*[1]; *Pipistrellus pipistrellus*[2,4]; *Rattus rattus*[3];	[1]Spagnesi, 1999b, 2002b; [2]Mucedda *et al.*, 2015; [3]Martin *et al.*, 2000; [4]Mucedda *et al.*, 2016;
La Maddalena	*Rinolophus ferrumequinum*[8]; *Pipistrellus pipistrellus*[1,8]; *Pipistrellus kuhli*[1,4,7,8]; *Pipistrellus pygmaeus*[8]; *Myotis capaccini*[8]; *Hypsugo savii*[8]; *Tadarida teniotis*[8]; *Lepus capensis*[3]; *Sus scrofa*[5]; *Rattus rattus*[6];	[1]Mucedda *et al.*, 2015; [2]CK map recorded in 1987; [3]Spagnesi, 1999a, 2002a; [4]MZUF-13036; [5]Pedrotti & Tosso, 2002; [6]Capizzi & Santini, 1999d, 2002d; [7]Zava et al. (1996); [8]Mucedda et al., 2016;
Santo Stefano	*Rhinolopus ferruequinum*[2]; *Pipistrellus pipistrellus*[1,2]; *Hypsugo savii*[2]; *Tadarida teniotis*[2]	[1]Mucedda *et al.*, 2015; [2]Mucedda *et al.*, 2015;
Caprera	*Rhinolophus ferrumequinum* [8]; *Rhinolopus hipposideros*[8]; *Pipistrellus pipistrellus*[7,8]; *Pipistrellus kuhlii*[8]; *Pipistrellus pygmaeus*[8]; *Myotis capaccini*[8]; *Hypsugo savii*[8]; *Tadarida teniotis*[8]; *Erinaceus europaeus*[1]; *Crocidura pachyura* [2,3]; *Oryctolagus cuniculus*[4]; *Sus scrofa*[5,6];	[1]Cossu *et al.*, 1994; [2]Amori & Masseti, 1996; [3]Thibault *et al.*, 1988; [4]Spagnesi, 1999b, 2002b; [5]Pedrotti & Toso, 1999; [6]Pedrotti & Tosso, 2002; [7]Mucedda *et al.*, 2015; [8]Mucedda et al., 2016;
Figarolo	*Pipistrellus pipistrellus*[1,7]; *Pipistrellus kuhlii*[7]; *Hypsugo savii*[7]; *Tadarida teniotis*[7]; *Ovis orientalis*[2,3,4,5,6];	[1]Mucedda *et al.*, 2015; [2] Pratesi & Tassi, 1973; [3]Ruiu, 1993; [4]Bocchieri & Satta, 1999; [5]Trainito, 2008; [6]Trainito & Navone, 2011; [7]Mucedda *et al.*, 2016;
Tavolara	*Suncus etruscus*[1]; *Rinolophus ferrumequinus*[2,20]; *Pipistrellus pipistrellus*[6,20]; *Pipistrellus kuhlii*[20]; *Miniopoterus schreibersi*[1,3,4,20]; *Hypsugo savii*[20]; *Myotis* sp.[20]; *Eptesicus serotimus*[20]; *Tadarida teniotis*[4,17,20]; †*Oryctolagus cuniculus*[5]; *Capra aegagrus*[6,7,8,9,10,11,12]; *Rattus norvegicus*[13,14,15]; *Rattus rattus*[14,15,16]; *Mus musculus*[17,18,19];	[1]Ranzi, 1971; [2]Grafitti & Mucedda, 1995; [2]Lanza & Agnelli, 1999a, 2002a; [3]Lanza & Agnelli, 1999b, 2002b; [4]Navone, 2014; [5]Pratesi & Tassi, 1971,1973; [6]Mucedda *et al.*, 2015; [6]Cetti, 1774; [7]Valery, 1837; [8]McGrigor, 1866; [9]Toschi, 1953 ; [10]De Beaux, 1955 ; [11]Couturier, 1959; [12]Masseti, 2002a, 2003a, 2008, 2009, and 2014; [13]Capizzi & Santini, 1999e, 2002e; [20] Sarà, 1998; [14]Trainito, 2008; [15]Trainito & Navone, 2011; [16]Capizzi & Santini, 1999d, 2002d; [17]Paolo Agnelli, *in verbis* 2016; [17]Baccetti *et al.*, 2009; [18]Sposimo *et al.*, 2012; [19]Ragionieri *et al.*, 2013; [20]Mucedda *et al.*, 2016;
Isola Piana off Tavolara	*Pipistrellus pipistrellus*[1,4]; *Pipistrellus kuhlii*[4]; †*Oryctolagus cuniculus*[2]; *Rattus rattus*[3]; *Mus musculus*[3];	[1]Mucedda *et al.*, 2015; [2]Trainito, 2008; [2]Trainito & Navone, 2011; [3]Paolo Sposimo, *in litteris* 2014; [4]Mucedda *et al.*, 2016;
Isola dei Cavalli	*Rattus rattus*[1]; *Mus musculus*[1];	[1]Paolo Sposimo, *in litteris* 2014;
Proratora	*Rattus rattus*[1]; *Mus musculus*[1];	[1]Paolo Sposimo, *in litteris* 2014;
Reuloino or Isolotto Rosso	*Mus musculus*[1];	[1]Paolo Sposimo, *in litteris* 2014;
Molarotto	†*Rattus rattus*[1];	[3]Paolo Sposimo, *in litteris* 2014;
Molara	†*Oryctolagus cuniculus*[2]; *Pipistrellus pipistrellus*[1,5]; *Hypsugo savii*[5]; *Tadarida teniotis*[05]; *Rattus rattus*[3,4];	[1]Mucedda *et al.*, 2015; [2] Dario Capizzi, *in litteris* 2014; [3]Ranzi, 1971; [4]Navone, 2014; [5]Mucedda *et al.*, 2016;
Serpentara	*Pipistrellus pipistrellus*[1,3]; *Tadarida teniotis*[3]; *Rattus rattus*[2];	[1]Mucedda *et al.*, 2015; [2]Capizzi & Santini, 1999d, 2002d;
Isola dei Cavoli	*Pipistrellus pipistrellus*[1,3]; *Pipistrellus kuhlii*[3]; *Vulpes vulpes*[2]; *Rattus rattus*[2]	[1]Mucedda *et al.*, 2015; [2]Scrugli & Cogoni, 1995; [3]Mucedda *et al.*, 2015;
Isola Rossa	*Oryctolagus cuniculus*[1];	[1]Spagnesi, 1999b, 2002b;
Vacca	*Oryctolagus cuniculus*[1]; *Rattus rattus*[2];	[1]Spagnesi, 1999b, 2002b; [2]Capizzi & Santini, 1999d, 2002d;
Toro	*Oryctolagus cuniculus*[1];	[1]Spagnesi, 1999b, 2002b;

Table 1/1. Terrestrial mammals of the satellite islands of Sardinia. MZUF: Museo di Zoologia "La Specola" dell'Università di Firenze; MCSNM: Museo Civico di Storia naturale di Milano.

island	species	references
Sant'Antioco	*Pipistrellus pipistrellus*[1]; *Myotis punicus*[2,3,4,5]; *Sus scrofa*[6 C];	[1]Mucedda *et al*., 2015; [2]MSNM 722; [3]Zava & Violani (1992); [4]Mucedda et al. 2016; [5]Zava et al. (1996); [6]present work;
Isolotto di Cala Vinagra	*Tadarida teniotis*[1];	[1]Fornasari *et al*, 1997;
San Pietro	*Erinaceus europaeus*[1] *Crocidura pachyura*[2]; *Rinolophus hipposideros*[1,3,4,10,12]; *Myotis capaccini*[12]; *Myotis punicus*[1,10,13]; *Pipistrellus pipistrellus*[1,3,5,6,10,11,12]; *Pipistrellus kuhli*[1,3,10,12]; *Tadarida teniotis*[1,3,6,10,12]; *Lepus capensis*[7]; *Oryctolagus cuniculus*[1,8]; †*Vulpes vulpes*?[1]; *Rattus rattus*[1,9];	[1] Zava *et al*., 1996[E]; [2]Sarà, 2008; [3]Fornasari *et al*, 1997; [4]Lanza & Agnelli, 1999c, 2002c; [5]Mocci Demartis & Secci, 1997; [6]Lanza & Agnelli, 1999d, 2002d; [5]Mocci Demartis & Secci, 1997; [6]Lanza & Agnelli, 1999e, 2002e; [7]Spagnesi, 1999a, 2002a; [8]Cetti, 1774; [8]Vallebona, 1974; [8]Racheli, 1981; [9]Capizzi & Santini, 1999d, 2002d; [10] Zava & Violani, 1992; [11]Mucedda *et al*., 2015; [12]Mucedda *et al*., 2016; [13]Skiba, 2009;
Mal di Ventre	*Oryctolagus cuniculus*[1]; *Rattus rattus*[2]; *Mus musculus*[2,3];	[1]Spagnesi, 1999b, 2002b; [2]Capizzi & Santini, 1999c, 2002c; [3]Andreotti *et al*., 2001;
Foradada		
Isola Piana, off Asinara	*Rattus rattus*[1]; *Sus scrofa*[2];	[1]Martin *et al*., 2000; [2]Bazzoni, 2013;
Asinara	*Erinaceus europaeus*[1]; *Suncus etruscus*[1,2,3,4]; *Crocidura pachyura*[1,3]; *Lepus capensis*[5,6]; *Rinolophus hipposideros*[25,30 B]; *Rinolophus ferrumequinum*[29,30]; *Myotis daubentoni*[30]; *Miniopterus schreibersi*[30]; *Pipistrellus pipistrellus*[28,30]; *Pipistrellus kuhlii*[30]; *Pipistrellus pygmaeus*[30]; *Hypsugo savii*[30]; *Eptesicus serotinus*[30]; *Nyctalus leisleri*[30]; *Tadarida teniotis*[30]; *Mustela nivalis*[1,3,7,9]; *Equus africanus*[10,11,12,13]; *Sus scrofa*[1,14,15,16]; *Capra aegagrus*[17,18,19,20,21]; *Ovis orientalis*[1,13]; *Apodemus sylvaticus*[3,22]; *Rattus norvegicus*[3,23,24]; *Rattus rattus*[26]; *Mus musculus*[1,3,26]; *Eliomys quercinus*[1,3,27];	[1]Torre & Monbailliu, 1993; [2]Pratesi & Tassi, 1973; [3]Amori & Masseti, 1996; [4]Contoli & Amori, 2008; [5]Segala, 1991; [6]Spagnesi, 1999a, 2002a; [7]Cossu *et al*., 1994; [8]Masseti, 1995; [9]De Marinis & Masseti, 2003; [10]Cherchi Paba, 1974; [11]Casu *et al*., 1989; [12]Pinna *et al*., 1990, and Pinna *et al*., 1993; [13]Massoli Novelli, 2003; [14]Torre & Monbailliu, 1993; [14]Pedrotti & Toso, 1999; [15]Pedrotti & Tosso, 2002; [16]Massoli Novelli, 2003; [17]De Beaux, 1955; [18]Masseti, 2008, 2009, 2014; [19]De Beaux, 1955 ; [20]Couturier, 1959; [21]Masseti, 2002a, 2003a, 2008, 2009, and 2014; [22]Capizzi & Santini, 1999a, 2002a; [23]Sarà, 1998; [24]Capizzi & Santini, 1999e, 2002e; [25]Winter et al., 2015; [26]Capizzi & Santini, 1999c, 2002c; [27]Capizzi & Santini, 1999b, 2002b; [28]Mucedda *et al*, 2015; [29]Bardi et al., 2014; [30]Mucedda *et al*., 2016;

Table 1/2. Terrestrial mammals of the satellite islands of Sardinia. MZUF: Museo di Zoologia "La Specola" dell'Università di Firenze; MCSNM: Museo Civico di Storia naturale di Milano.

mouse-eared bat, *M. punicus* Felten, Spitzenberger et Storch, 1977, recorded by Skiba (2009) from Sant'Antioco.

CONCLUSIONS

According to the data collected in the present study the terrestrial mammalian fauna of the circum-Sardinian islands amounts to 28 species, including 13 bats (*Rhinolophus ferrumequinum, Rhinolopus hipposideros. Myotis capaccini. Myotis daubentoni, Myotis punicus, Pipistrellus pipistrellus, Pipistrellus kuhli, Pipistrellus pygmaeus, Miniopterus schreibersi, Hypsugo savii, Eptesicus serotinus, Nyctalus leisleri,* and *Tadarida teniotis*) and 15 non-volant mammals (*Erinaceus europaeus, Crocidura pachyura, Suncus etruscus, Lepus capensis, Oryctolagus cuniculus, Vulpes vulpes, Mustela nivalis, Sus scrofa, Capra aegagrus, Ovis orientalis, Apodemus sylvaticus, Rattus norvegicus, Rattus rattus, Mus musculus,* and *Eliomys quercinus*) (Table 1). Today, a part from the bats, the faunistic horizons of all these islands are no more characterised by endemic Pleistocene species. The exclusive present occurrence of non-volant continental mammals on the satellite islands of Sardinia seems to be linked essentially to the introduction by man during the Holocene.

ACKNOWLEDGEMENTS

Among the many friends and colleagues who helped me as I was preparing this paper, I would like to express my appreciation and gratitude in par-

ticular to Paolo Agnelli, Museo di Zoologia "La Specola" dell'Università di Firenze (Italy); Dario Capizzi, Direzione Regionale Ambiente e Sistemi Naturali, Regione Lazio (Italy); Paolo Sposimo, NEMO Nature Environment Management Operators, Firenze (Italy), and Bruno Zava, Wilderness Studi Ambientali, Palermo (Italy). Special thanks are due to Mauro Mucedda, Gruppo Italiano Ricerca Chirotteri (Sassari, Italy), for having set at my disposal the results of the study carried out on the bats of the small islands of Sardinia.

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Biodiversity Journal, 2019, 10 (4): 383–388 https://doi.org/10.31396/Biodiv.Jour.2019.10.4.383.388
MONOGRAPH

Effects of *Caulerpa cylindracea* Sonder (Chlorophyta Caulerpaceae) on marine biodiversity

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ABSTRACT The chief purpose of Marine Protected Areas (MPAs) is biodiversity conservation. The effects that invasive alien species (IAS) have on MPAs are not yet fully known, even though assessing them is fundamental. Effective management plans, indeed, also require knowledge on the distribution, spread dynamics and impact of IAS. We report first observations on the effects of *Caulerpa cylindracea* Sonder (Chlorophyta Caulerpaceae) on the communities living along the coasts of the Island of Favignana (Egadi Islands MPA, Sicily, Italy). We found that *C. cylindracea* may have negative effects on the habitat where it settles in two different ways: a) affecting the structure of the native algal community which presents a low diversity, and b) favouring the settlement of other alien species such as *Branchiomma bairdi* (McIntosh, 1885) (Polychaeta Sabellidae).

KEY WORDS Biodiversity; *Branchiomma bairdi; Caulerpa cylindracea*; alien species; Mediterranean Sea.

Received 04.07.2019; accepted 20.08.2019; published online 20.12.2019
Proceedings of the 4th International Congress on Biodiversity "Man, Natural Habitats and Euro-Mediterranean Biodiversity", November 17th-19th, 2017 - Malta

INTRODUCTION

The Mediterranean Sea is an important hotspot for non-indigenous species (NIS, i.e. organisms introduced outside of their natural, past or present, range and outside of their natural dispersal potential). NIS may in time become invasive (i.e. invasive alien species "IAS") and may cause biodiversity loss and ecosystem service changes (Brunel et al., 2013; Giakoumi, 2014; Vergés et al., 2014, 2016). IAS are recognised as one of the most serious threats, after habitat losses, to biodiversity and natural ecosystem functioning. In the Mediterranean Sea, due to multiple human-related stressors, the number of recorded NIS has enormously increased in the last 100 years (Occhipinti-Ambrogi et al., 2011a, b; Katsanevakis et al., 2014), reaching about 1000 NIS, of which 134 species are macrophytes (Verlaque et al., 2015; Alós et al., 2016).

Among IAS, *Caulerpa cylindracea* Sonder 1845 (Chlorophyta Caulerpaceae) (until Belton et al., 2014 it was reported in the literature as *Caulerpa racemosa* var. *cylindracea* (Sonder) Verlaque, Huisman et Boudouresque 2003), introduced from Australia and New Caledonia (Belton et al., 2014) and reported for the first time in Italy in 1993 (in Sicily at Baia di San Panagia and at the Island of Lampedusa, Alongi et al., 1993), has raised serious concern due to its ascertained impact on Mediterranean communities (Boudouresque et al., 1995; Antolić et al., 2008; Klein & Verlaque, 2008;

Piazzi & Balata, 2008; Papini et al., 2013; Katsanevakis et al., 2014).

Sicily and smaller surrounding Islands (including Marine Protected Areas "MPA"), located at the crossroads between the eastern and western sectors of the Mediterranean Sea and characterized by intense maritime traffic, are particularly vulnerable and suitable to biological marine invasions (Occhipinti-Ambrogi et al., 2011a, b; Coll et al., 2012; Papini et al., 2013; Katsanevakis et al., 2014). To plan effective management and conservation strategies, reliable data on distribution, spread dynamics and impacts of IAS are essential. For this reason, regular monitoring and surveillance programs are strongly needed. Since intensive monitoring programs could be very expensive, citizen science, involving citizens (e.g. tourists, fishermen, divers) in the collection of data, could be a useful tool for providing data on IAS that would otherwise be impossible to collect because of limitations on time and resources (Mannino & Balistreri, 2018).

We report first observations on the effects of *C. cylindracea* on the communities living along the coasts of the Island of Favignana (Egadi Islands MPA), carried out during the citizen science project '*Caulerpa cylindracea* - Egadi Islands', aimed at monitoring the spread dynamics of *C. cylindracea* within the Egadi Islands MPA.

MATERIAL AND METHODS

Study area

The Egadi Islands MPA (Aegadian Archipelago), instituted in 1991, is the largest Italian MPA. This archipelago, located approximately 7–9 km from the western coast of Sicily (Italy, Tyrrhenian Sea), is composed of three main islands (Favignana, Marettimo and Levanzo) and a few small islets (Galeotta, Galera, Preveto, Formica and Maraone).

The study was carried out at Cala San Giuseppe, one of the old calcarenitic opencast mine, currently submerged by the sea, located in the northern side of the Favignana Island (37°56'07.00''N, 12°20'02.59''E - Fig. 1).

Sampling

Samples were carried out in summer 2016 in two areas (Area 1, Area 2; see Fig. 1), characterized by different sedimentation and hydrodynamic conditions. The different environmental conditions are essentially linked to the presence (Area 1) or absence (Area 2) of calcarenitic blocks responsible for the reduction of the hydrodynamism and the increase of the sediment accumulation.

Within each area, two sites were selected, one characterized by a high coverage of *C. cylindracea* and the other one by the presence of a few thalli of the alga. At each site six replicated 400 cm^2 quadrats were placed in order to estimate the mean abundance values of the all recorded taxa.

RESULTS

Significant differences between the two areas were observed whereas no remarkable differences were highlighted between the two sites of each area. In the area 1, characterized by a higher rate of sedimentation, *C. cylindracea* was more abundant (a mean % coverage of 46±8.1) and behaved as a pioneer species (Fig. 2). The active mechanism of stolonisation allowed *C. cylindracea* to

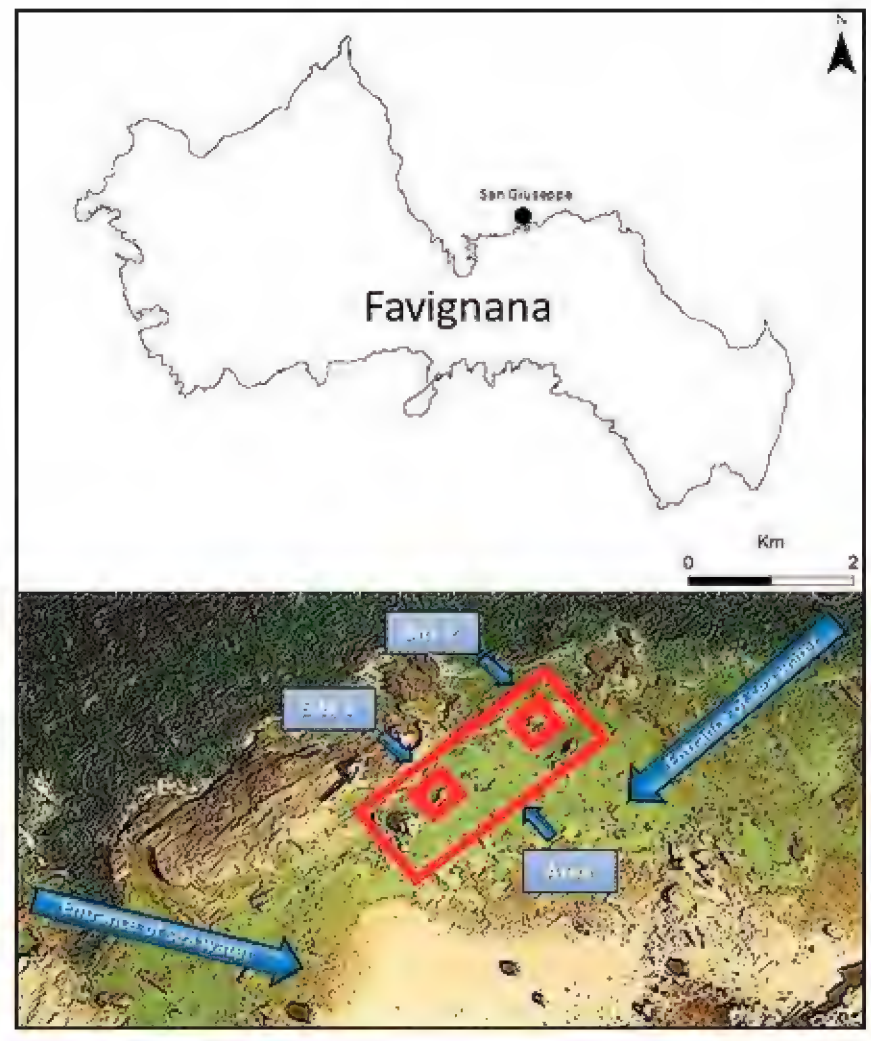

Figure 1. Map showing Cala San Giuseppe (Favignana Island, Sicily, Italy) and the sampling areas.

Figures 2–5. Area 1: multi-layered mats formed by *Caulerpa cylindracea* stolons (Fig. 2), *Branchiomma bairdi* among patches of *C. cylindracea* (Fig. 3); Area 2: the sponge *Chondrilla nucula* (Figs. 3, 4).

spread rapidly, forming compact multi-layered mats which trap the sediment and host native macroalgae, growing strictly intermingled to *C. cylindracea* stolons. In this area the diversity of the algal community was not particularly high (see Table 1). The algal community was essentially dominated by red and green algae such as *Jania rubens* (Linnaeus) J.V. Lamouroux, 1816 (Rhodophyta Corallinaceae) (a mean % coverage of 38±6.8), *Cladophora prolifera* (Roth) Kützing, 1843 (Chlorophyta Cladophoraceae) (a mean % coverage of 10±1.4), and *Laurencia dendroidea* J. Agardh, 1852 (Rhodophyta Rhodomelaceae) (a mean % coverage of 10±4.5). The mats, entrapping sediments, also favoured the establishment of another alien species, *Branchiomma bairdi* (McIntosh, 1885), a tropical tube-building sabellid polychaete, among patches of *C. cylindracea* (Fig. 3). In all 114 individuals were recorded, mainly concentrated (88 individuals) where *C. cylindracea* formed consistent mats entrapping a huge quantity of sediment.

Instead, sponges, particularly *Chondrilla nucula* Schmidt, 1862 (Spugna Nocciolina, a mean % coverage of 50±4.5), take advantage of the conditions in the area 2, characterized by a low rate of sedimentation, occupying quite all the available substrate (Figs. 4, 5). In this area a few thalli of *C. cylindracea* and low coverage values of the native macroalgae were observed (see Table 2). *Jania rubens* (a mean % coverage of 30±4.1) was the dominant species, mainly growing as epiphyte.

TAXA	COVERAGE (%)
Caulerpa cylindracea	46±8.1
Jania rubens	38±6.8
Cladophora prolifera	10±1.4
Laurencia dendroidea	10±4.5
Anadyomene stellata	5±1.5
Lychaete pellucida	5±1.7
Dictyota dichotoma	1±0.8
Flabellia petiolata	1±0.7
Padina pavonica	1±0.5

Table 1. Area 1: coverage values (mean ± SE, n = 6) of *Caulerpa cylindracea* and the native macroalgae.

TAXA	COVERAGE (%)
Chondrilla nucula	50±4.5
Jania rubens	30±4.1
Laurencia dendroidea	5±0.6
Caulerpa cylindracea	2±0.8
Padina pavonica	1±0.5

Table 2. Area 2: coverage values (mean ± SE, n = 6) of *Chondrilla nucula*, *Caulerpa cylindracea* and the native macroalgae.

DISCUSSION AND CONCLUSIONS

First results showed how the rate of sedimentation has an important role in structuring the communities in the studied areas. In the area 1 we assisted to a cascade process. Precisely, the high rate of sedimentation favoured the growth and spread of *C. cylindracea* which in turn allowed a further increase of sedimentation, as consequence of the multi-layered mats trapping the sediment, which favoured the establishment of several individuals of *Branchiomma bairdi* among the patches of *C. cylindracea*. This biofouler worm, already recorded at the Egadi Island MPA (Mytilineou et al., 2016), clearly takes advantage of the additional debris among the stolons of *C. cylindracea*, on which it can easily settle. Even though this worm was originally described from Bermuda and Caribbean Sea (western Atlantic), its origin is currently unknown (Ramalhosa et al., 2014).

In the area 2, instead, where the rate of sedimentation is low, sponges such as *Chondrilla nucula*, take advantage behaving as pioneer species and they also maintain low both the rate of sedimentation and the biodiversity. Indeed, they do not allow algal species, *C. cylindracea* included, to settle well in the area, as clearly shown by the low coverage values observed for *C. cylindracea* and the native macroalgae. The low rate of sedimentation does not allow also the settlement of *Branchiomma bairdi* individuals, which do not find suitable environment conditions for their establishment. Therefore, *C. cylindracea* may have negative effects on the habitat where it settles in two different ways: a) affecting the structure of the native algal community which presents a low diversity, and b) favouring the settlement of other alien species. It was already observed by Baldacconi & Corriero (2009) how *C. cylindracea* (reported as *C. racemosa* var. *cylindracea*) may significantly affect the percentage cover of sponge assemblage of coralligenous concretions, likely due to the ability of *C. cylindracea* to overgrow several sponge species.

Since Sicily and the circum-Sicilian Islands, are vulnerable to biological marine invasions, regular monitoring programs are needed to assess the spread dynamics of invasive species, particularly in MPAs such as Egadi islands MPA. In the MPAs, high rates of visitation could promote the introduction of invasive species through increased disturbance and vectors (e.g., boat anchors, SCUBA equipment, hull fouling) and subsequent dispersal of propagules (Britton-Simmons & Abbott, 2008; Burfeind et al., 2013), therefore an IAS strategy integrated into the management plan of the Egadi Islands MPA may be highly desirable.

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Biodiversity Journal, 2019, 10 (4): 389–404 https://doi.org/10.31396/Biodiv.Jour.2019.10.4.389.404
MONOGRAPH

The Mediterranean Sepiolidae (Mollusca Cephalopoda) diversity

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ABSTRACT Sepiolidae (Mollusca Cephalopoda) is the most diverse cephalopod family in the Mediterranean Sea, where 17 species of this taxon have been identified. In the present review, the updated list of the Mediterranean sepiolids is given along with the species type locality, the first documented record in this basin and the present geographical distribution. The exclusion of *Sepiola atlantica* and *Heteroteuthis atlantis* - species that have been reported in the Mediterranean but whose presence is not warranted - from the list is also explained. Moreover, patently erroneous information about the bathymetric and geographical distributions of *Sepiola rondeletii* is revised. The extreme rarity of *Sepiola aurantiaca* and the comparatively recent entrance into the Mediterranean and establishment of *Stoloteuthis leucoptera* are discussed. The genesis of the Mediterranean sepiolid-fauna, in relation to the NE Atlantic fauna, is dealt with and, in particular, the reasons that determined its comparatively broad diversity are examined. They are to be traced back to both the mode of life and reproductive biology of these small-sized cephalopods. In connection with the latter matter, the high degree of endemism in the subfamily Sepiolinae is also explained.

KEY WORDS Cephalopoda; biogeography; biodiversity; Mediterranean; NE Atlantic Ocean.

Received 20.10.2018; accepted 19.01.2019; published online 20.12.2019
Proceedings of the 4th International Congress on Biodiversity "Man, Natural Habitats and Euro-Mediterranean Biodiversity", November 17th-19th, 2017 - Malta

To Adolf Naef (1883-1949)
and George Evelyn Hutchinson (1903-1991),
perennial sources of inspiration

INTRODUCTION

Sepiolidae is the most diverse cephalopod family in the Mediterranean Sea, which represents a hot-spot for this taxon since its maximum worldwide richness value was recorded just in this ecoregion (Rui et al., 2019). In all, 17 sepiolid species have been identified in the Mediterranean (Bello, 2008, 2017); all of them are small-sized. They are assigned to three subfamilies, namely Sepiolinae, Rossiinae and Heteroteuthinae, which are distributed worldwide. Members of a fourth, undescribed subfamily were discovered in the Pacific Ocean (Young, 2007).

The present review aims, firstly, at establishing the critical systematic list of the Mediterranean members in this family; secondly, at discussing its biogeography in the north-eastern Atlantic and Mediterranean districts.

To the former aim, the first documented record in this sea is reported for each species and the un-

warranted, questionable records, both geographically- and depth-wise, are discussed. In fact, the members of Sepiolinae are not promptly identifiable to the species level, especially before their sexual maturation, hence the literature, chiefly the old one, is crowded with many determination mistakes. In this respect, we have to mention that the first scholar to understand the systematics of Sepiolidae, was Adolf Naef (1883-1949). He, when working at the Stazione Zoologica in Naples, revised the whole Mediterranean teuthofauna (Naef, 1923) and described two new genera and seven new species of Sepiolinae (Naef, 1912a, 1912b, 1912c, 1916).

Identification tools for the Mediterranean Sepiolidae are Naef (1923) (a monograph of paramount importance), Mangold & Boletzky (1987), Guerra (1992) (this also includes NE Atlantic taxa), Bello (1995), Reid & Jereb (2005) (this is a reference worldwide review that includes the Atlantic species), Bello (2013) (this only relates to the Atlantic-Mediterranean *Sepiola atlantica* group).

As for the latter aim, the biogeography of the Mediterranean Sepiolidae was discussed by Bello (2003). In the present paper, it is further considered in relation to the NE Atlantic sepiolid-fauna in order to explain the genesis of the Mediterranean sepiolid diversity.

MATERIAL AND METHODS

This review is based on the relevant literature. In particular, I referred to the "Checklist of the flora and fauna in Italian seas" (Bello, 2008, 2017) for the systematic inventory of the Mediterranean Sepiolidae species, Bello (2015) for their updated nomenclature, and Reid & Jereb (2005) for their general distribution in both the Mediterranean Sea and the Atlantic Ocean.

Following the critical systematic list of the Mediterranean Sepiolidae, each specific entity is reported with its type locality, first documented Mediterranean record and general distribution. Next, peculiar cases are dealt with: the unwarranted Mediterranean and Atlantic records, the recent entrance into the Mediterranean, and the occurrence in this sea of rare sepiolid species.

RESULTS

Systematic list of Mediterranean Sepiolidae

In each subfamily and genus, the type genus and type species, respectively, are reported first, followed by the remaining genera and species arranged in alphabetical order.

Familia SEPIOLIDAE Leach, 1817
Subfamilia SEPIOLINAE Leach, 1817

Sepiola Leach, 1817
- *Sepiola rondeletii* Leach, 1817
- *Sepiola affinis* Naef, 1912
- *Sepiola aurantiaca* Jatta, 1896
- *Sepiola boletzkyi* Bello et Salman, 2015
- *Sepiola bursadhaesa* Bello, 2013
- *Sepiola intermedia* Naef, 1912
- *Sepiola ligulata* Naef, 1912
- *Sepiola robusta* Naef, 1912
- *Sepiola steenstrupiana* Levy, 1912

Rondeletiola Naef, 1921
- *Rondeletiola minor* (Naef, 1912)

Sepietta Naef, 1912
- *Sepietta oweniana* (d'Orbigny in Férussac et d'Orbigny, 1841)
- *Sepietta neglecta* Naef, 1916
- *Sepietta obscura* Naef, 1916

Subfamilia HETEROTEUTHINAE Appellöf, 1898

Heteroteuthis Gray, 1849
- *Heteroteuthis dispar* (Rüppell, 1844)

Stoloteuthis Verrill, 1881
- *Stoloteuthis leucoptera* (Verrill, 1878)

Subfamilia ROSSIINAE Appellöf, 1898

Rossia Owen, 1835
- *Rossia macrosoma* (delle Chiaje, 1830)

Neorossia Boletzky, 1971
- *Neorossia caroli* (Joubin, 1902)

The Mediterranean Sepiolidae

Sepiolinae

The nominotypical subfamily, Sepiolinae, is the best-defined taxon in this family. In addition to

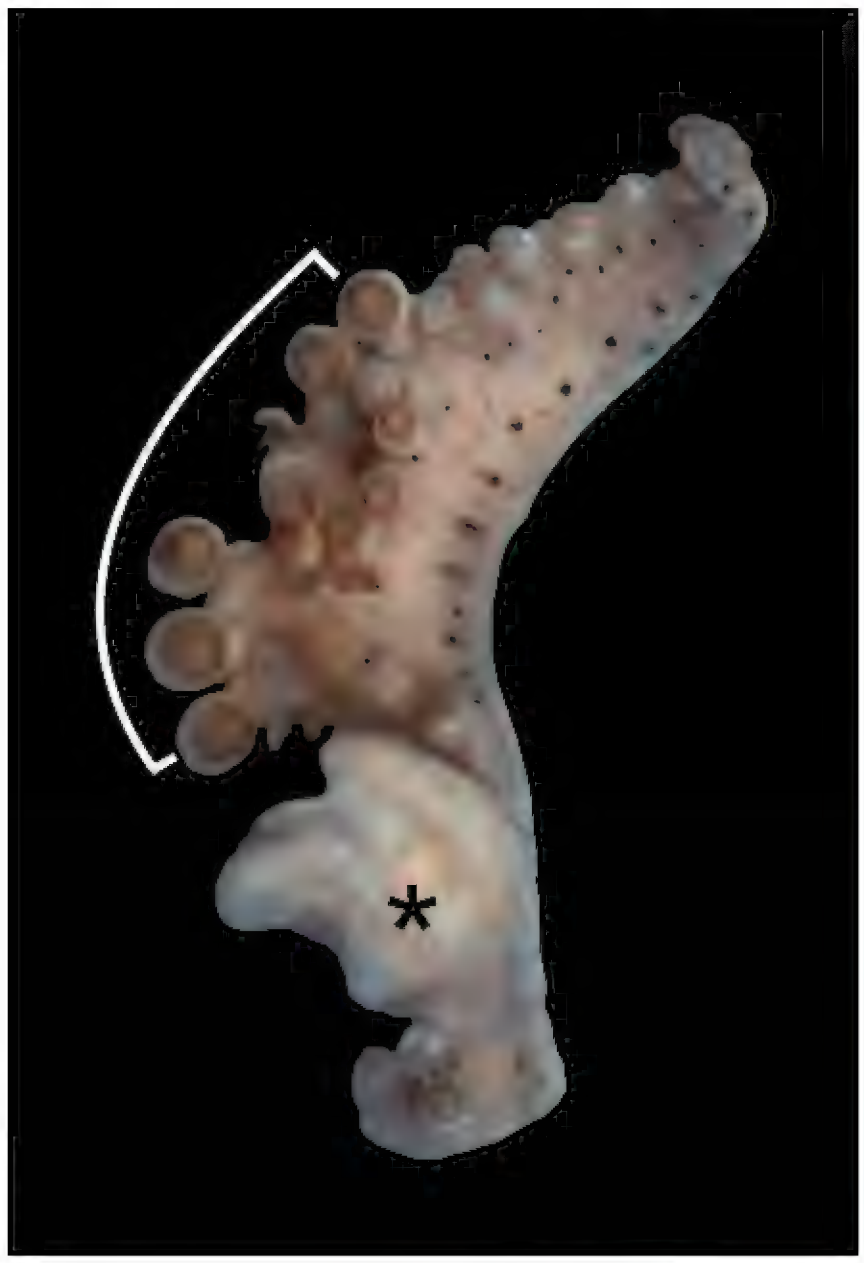

Figure 1. Hectocotylus of *Sepiola boletzkyi*. The left dorsal arm of Sepiolinae bears modified suckers: the asterisk * marks a group of suckerless lengthened stalks, the white line points out several enlarged suckers in the dorsal row.

Figure 2. Visceral organs of a female *Sepiola boletzky*. The white line encompasses the bursa copulatrix, which is the terminal part of the gonoduct; on it the male implants its spermatophores during copulation.

other features, it is characterized by peculiar copulatory organs: the hectocotylus in males, i.e. the left dorsal arm modified to transfer spermatophores to females (Fig. 1) and the bursa copulatrix in females, placed in the left ventral mantle cavity (Fig. 2). The latter is an apomorphic character, a unique feature of Sepiolinae. This taxon contains the genera *Sepiola* (type genus), *Euprymna, Inioteuthis*, *Rondeletiola* and *Sepietta*. *Euprymna* and *Inioteuthis* are exclusively Indo-Pacific. Members of this taxon are mainly benthic.

Sepiola rondeletii

Type locality. "European sea" (Leach, 1817); corrected type locality: Mediterranean Sea (Sweeney, 2001; Bello, 2015). First Mediterranean documented record: Naef (1912a). Note that almost all European sepioline species were ascribed to the nominal species *Sepiola rondeletii* before the revision by Naef (1912a) (see further), so that it is virtually impossible to validate all records of this sepioline published before Naef's works (1912a, 1912b, 1923), unless supported by museum specimens and/or well-defined illustrations. Naef (1912a) was the first author to accurately define this species, hence, the first trustworthy identifications are to be credited to him (Bello, 2015).

Distribution and Ecology. It is a quasi-endemic Mediterranean sepioline, which is distributed all over the Mediterranean Sea (Reid & Jereb, 2005) and in the Atlantic Ocean close to the Strait of Gibraltar, e.g. in the Gulf of Cadiz (Guerra, 1982) (quasi-endemic sensu Bello (2003) is a Mediterranean species that is also found in the Atlantic Ocean in the vicinities of Gibraltar). Although *Sepiola rondeletii* has been reported in the northeast-

ern Atlantic Ocean from the North Sea to Senegal (see review by Reid and Jereb, 2005), the northern Atlantic records are inaccurate according to Groenenberg et al. (2009). The typical habitat of this sepioline is "*sandy and muddy substrates, common in* Posidonia *seagrass beds down to 35 m*" (Reid & Jereb, 2005: 168), however deeper finds, down to 450 m depth, are reported in the literature (cf. Reid & Jereb, 2005). In the Gulf of Cadiz, two specimens were collected from a muddy bottom 190 m deep (Guerra, 1982). See the next chapter "Unwarranted sepiolid records" for geographically- or depth-wise improbable records of *Sepiola rondeletii.*

Sepiola affinis

TYPE LOCALITY. Gulf of Naples, Tyrrhenian Sea, western Mediterranean. First Mediterranean documented record: Naef (1912b). It proceeds from the original description.

DISTRIBUTION AND ECOLOGY. A Mediterranean endemic species. It is a shallow water sepioline that has been reported from the whole coastal belt of the western basin and from the northern coasts of the eastern basin (Reid & Jereb, 2005), but most probably it also lives along the northern African and Asia Minor shores.

Sepiola aurantiaca

TYPE LOCALITY. Gulf of Naples, Tyrrhenian Sea, western Mediterranean. First Mediterranean documented record: Jatta (1896). It proceeds from the original description.

DISTRIBUTION AND ECOLOGY. The only known specimens were collected in the Amontatura (or Ammontatura) channel (100 to 140 m deep) in the Gulf of Naples.

Sepiola boletzkyi

TYPE LOCALITY. Gulf of Ildir (Turkey), Aegean Sea, eastern Mediterranean. First Mediterranean documented record: Bello & Salman (2015). It proceeds from the original description.

DISTRIBUTION AND ECOLOGY. A shallow water species only known from its type locality.

Sepiola bursadhaesa

TYPE LOCALITY. Catalan Sea, western Mediterranean. First Mediterranean documented record: Bello (2013). It proceeds from the original description.

DISTRIBUTION AND ECOLOGY. It is only known from its type locality. It is most probably a shallow water species.

Sepiola intermedia

TYPE LOCALITY. Gulf of Naples, Tyrrhenian Sea, western Mediterranean. First documented record: Naef (1912a). It proceeds from the original description.

DISTRIBUTION AND ECOLOGY. *Sepiola intermedia* is a Mediterranean quasi-endemic, since it was also collected in the Gulf of Cadiz (West of Gibraltar) by Guerra (1982). Reid & Jereb (2005) refer that it is distributed, between 60 and 200 m of depth, in the whole Mediterranean except the Libyan, Egyptian and Asia Minor coasts. Anyway, most probably it also lives there.

Sepiola ligulata

TYPE LOCALITY. Gulf of Naples, Tyrrhenian Sea, western Mediterranean. First Mediterranean documented record: Naef (1912a). It proceeds from the original description.

DISTRIBUTION AND ECOLOGY. This is a comparatively deep water sepioline, living on muddy bottoms from a few tens of metres down to the upper slope (ca. 350 m). It was deemed a Mediterranean quasi-endemic (Bello, 2003) because the northernmost record was that by Guerra (1986) in the Ría de Vigo (NW Spain). The presence of a *Sepiola ligulata* population in that district was recently corroborated by the collection of paralarvae (Olmos-Pérez et al., 2017). Indeed, in addition to the whole Mediterranean Sea, it inhabits a wide portion of the NE Atlantic Ocean (de Heij et al., 2017), hence it cannot any longer be termed quasi-endemic.

Sepiola robusta

TYPE LOCALITY. Gulf of Naples, Tyrrhenian Sea, western Mediterranean. First Mediterranean docu-

mented record: Naef (1912a). It proceeds from the original description.

DISTRIBUTION AND ECOLOGY. An endemic Mediterranean found all over the basin, from a few tens of metres down to 500 m.

Sepiola steenstrupiana

TYPE LOCALITY. Off Villefranche (Alpes Maritimes, France), Gulf of Lions, western Mediterranean. First Mediterranean documented record: Levy (1912). It proceeds from the original description.

DISTRIBUTION AND ECOLOGY. It has been recorded in the northern part of the Mediterranean Sea and in the Levant Sea (Reid & Jereb, 2005). Outside the Mediterranean, it was collected in the Red Sea (Adam, 1973) and off the eastern Somali coast (Indian Ocean) (Rocha et al., 1998). Accordingly, *Sepiola steenstrupiana* is an endemic Mediterranean sepioline that seemingly crossed the Suez Canal in a counter-Lessepsian migration.

Rondeletiola minor

TYPE LOCALITY. Gulf of Naples, Tyrrhenian Sea, western Mediterranean. First Mediterranean documented record: Naef (1912a). It proceeds from the original description.

DISTRIBUTION AND ECOLOGY. This is a deep-water East Atlantic-Mediterranean sepioline. Reid & Jereb (2005) report that it is found from 76 to 496 m of depth all over the Mediterranean Sea, including the Sea of Marmara, and off the West African and West Iberian coasts, from Namibia to the Gulf of Biscay. A recent survey collected very many specimens farther North to the West of Ireland, and few scattered ones off Scotland and Norway, in a wider depth range, from 25 to 800 m (de Heij et al., 2017. *Rondeletiola minor* is one of the most frequently netted sepiolines in the Mediterranean Sea.

Sepietta oweniana

TYPE LOCALITY. Unknown. This species occurs in both the Mediterranean Sea and the NE Atlantic Ocean. First Mediterranean documented record: Jatta (1896). This author lumped in his description of "*Sepiola rondeletii*" several sepioline species from the Gulf of Naples, comprising *Sepietta* spp. too. The pictured elements of the purported *Sepiola rondeletii* include a hectocotylus (Jatta, 1896: pl. 14 fig. 28) (Fig. 3), which is unmistakably *Sepietta oweniana*'s (compare Jatta's figure to Naef, 1912b: fig. 1, shown in figure 6 of present paper).

DISTRIBUTION AND ECOLOGY. A NE Atlantic-Mediterranean species from Mauritania to North Norway (Reid & Jereb, 2005). The occurrence of a specimen farther North, in the SW Barents Sea, is possibly due to the ocean waters warming (Golikov et al., 2014). *Sepietta oweniana* is distributed over a wide depth range, from few tens of metres to over 1,000 m (Reid & Jereb, 2005), and is the most abundantly collected Mediterranean sepioline.

Sepietta neglecta

TYPE LOCALITY. Gulf of Naples, Tyrrhenian Sea, western Mediterranean. First Mediterranean documented record: Naef (1916). It proceeds from the original description.

DISTRIBUTION AND ECOLOGY. Reid & Jereb (2005) refer that *Sepietta neglecta* is found from 25 to 475 m of depth, in the whole Mediterranean, including the Sea of Marmara, and in the NE Atlantic Ocean from Marocco to the Orkney Islands and South Norway. De Heij et al. (2017) extended its distribution to the waters West of England, Wales and Ireland.

Sepietta obscura

TYPE LOCALITY. Gulf of Naples, Tyrrhenian Sea, western Mediterranean. First Mediterranean documented record: Naef (1916). It proceeds from the original description.

DISTRIBUTION AND ECOLOGY. A quasi-endemic Mediterranean sepioline that was found in most of the Mediterranean littoral waters and off the West Iberian coast (Reid & Jereb, 2005). According to the literature data referred by the same Authors, *Sepietta obscura* depth range is 27 to 376 m. In my opinion, this is a shallow water species that dwells on littoral grounds only, from few metres of depth on (personal observations).

HETEROTEUTHINAE

The taxon Heteroteuthinae is indeed polyphyletic (Allcock et al., 2014) and needs a thorough revision. Its members are characterized by a broad web between arms and a comparatively large mantle with extended ventral shield, which characters are indicative of their pelagic mode of life. Some of them display an occipital band, i.e. a dorsal commissure between mantle and head, a feature shared with Sepiolinae, e.g. *Heteroteuthis*, and others do not, which is typical of Rossiinae, e.g. *Stoloteuthis*. In addition to the type genus, *Heteroteuthis*, this subfamily includes *Amphorateuthis*, *Nectoteuthis*, *Iridoteuthis*, *Sepiolina*, and *Stoloteuthis* (Young et al., 2015).

Heteroteuthis dispar

TYPE LOCALITY. Off Messina, Strait of Messina, Mediterranean. First Mediterranean documented record: Rüppell (1844). It proceeds from the original description.

DISTRIBUTION AND ECOLOGY. A mesopelagic species that lives in the whole Mediterranean and the North Atlantic Ocean, down to about 1,600 m of depth (Reid & Jereb, 2005). It is very abundant and, despite its diminutive size, is a key item in food webs (e.g. Bello, 1999).

Stoloteuthis leucoptera

TYPE LOCALITY. Gulf of Maine, NW Atlantic Ocean. First Mediterranean documented record: Orsi Relini & Massi (1991), in the Ligurian Sea, western Mediterranean.

DISTRIBUTION AND ECOLOGY. Jereb & Reid (2005) refer that it is an amphi-Atlantic species living in the northern West Atlantic Ocean, from Canada to Florida, and from the Bay of Biscay to Namibia in the East Atlantic Ocean; depth range: 160-700 m. It entered the western Mediterranean Sea in recent times and established there a viable population (see further).

ROSSIINAE

The subfamily Rossiinae contains the largest-sized members of the Sepiolidae (up to 10 cm mantle length), which are characterized by the absence of both the occipital band and the ventral mantle shield. This subfamily includes the genera *Rossia* (type genus), *Austrorossia*, *Neorossia*, and *Semirossia*. All rossiines are benthic (Young & Vecchione, 2014).

Rossia macrosoma

TYPE LOCALITY. In the surroundings of Naples, Tyrrhenian Sea, western Mediterranean (Bello, 2015). First Mediterranean documented record: delle Chiaje (1830). It proceeds from the original description.

DISTRIBUTION AND ECOLOGY. A North Atlantic-Mediterranean sepiolid living on comparatively deep grounds, from 25 to 900 m (Reid & Jereb, 2005; de Heij et al., 2017). It dwells in the whole Mediterranean basin and in the Atlantic Ocean from Greenland to Norway and far south to Senegal (Reid & Jereb, 2005).

Neorossia caroli

TYPE LOCALITY. The Azores, North Atlantic Ocean. First Mediterranean documented record: Dieuzeide (1959), off the Aguelli island (Algeria), south-western Mediterranean.

DISTRIBUTION AND ECOLOGY. *Neorossia caroli* is found in the eastern Atlantic Ocean from Namibia to Iceland (Reid & Jereb, 2005). According to the review of these Authors, it is a typical upper slope benthic sepiolid recorded down to 1744 m; it has been recorded from neritic grounds as shallow as 40 m, although, in the Mediterranean Sea, the preferred upper limit is at about 400 m of depth.

Unwarranted sepiolid records

Adolf Naef, who may be rightly termed the father of modern Teuthology, revised several cephalopod taxa during his stay at the Stazione Zoologica of Naples. He did a most accurate study of Sepiolidae, whose taxonomic situation was quite muddy at that time, and described two new genera and seven new species (Naef, 1912a; 1912b; 1916; 1921; 1923). Before Naef's revision, all or almost all European sepioline species were ascribed to the nominal species *Sepiola rondeletii*

(cf. Bello, 2015). Hence, all records of "*Sepiola rondeletii*" prior to 1912 are to be regarded as unwarranted and should not be taken into account. For instance, the drawing of the purported hectocotylus of "*Sepiola Rondeleti*" presented by Joubin (1902: fig. 2) is evidently copied from Jatta (1896: pl. 14 fig. 28), whose figure, as reported above, depicts the hectocotylus of *Sepietta oweniana*, a species well established at that time (Fig. 4) (compare Joubin's figure to Jatta, loc. cit.). Also, the arm crown of "*Sepiola rondeletii*" depicted by Pfeffer (1908: fig. 56) is in fact misidentified: it belongs indeed to *Sepiola intermedia,* as Naef (1923) already showed; in Pfeffer's partial defence one may say that *Sepiola intermedia* had not yet been described when he wrote his paper. Sadly enough, even after Naef disclosed and described the Atlantic-Mediterranean diversity in Sepiolinae, many misidentifications have occurred (Bello, 2015). As reported above, in the current literature, the *Sepiola rondeletii* distribution is reported to cover, in addition to the Mediterranean Sea, the north-eastern Atlantic Ocean from the North Sea to Senegal (see review by Reid & Jereb, 2005). However, later on, Groenenberg et al. (2009) suggested that the northern Atlantic records are inaccurate. Also, some depth records, as deep as 450 m (cf. Reid & Jereb, 2005), are questionable since this sepioline is typically a coastal cephalopod.

As for comparatively recent implausible records, Würtz et al. (1995) reported a finding of *Sepiola atlantica* in the South Tyrrhenian Sea, which was not warranted in any way, i.e. no textual description, no photograph, no deposit in any institutional collection. A search for the specimen from

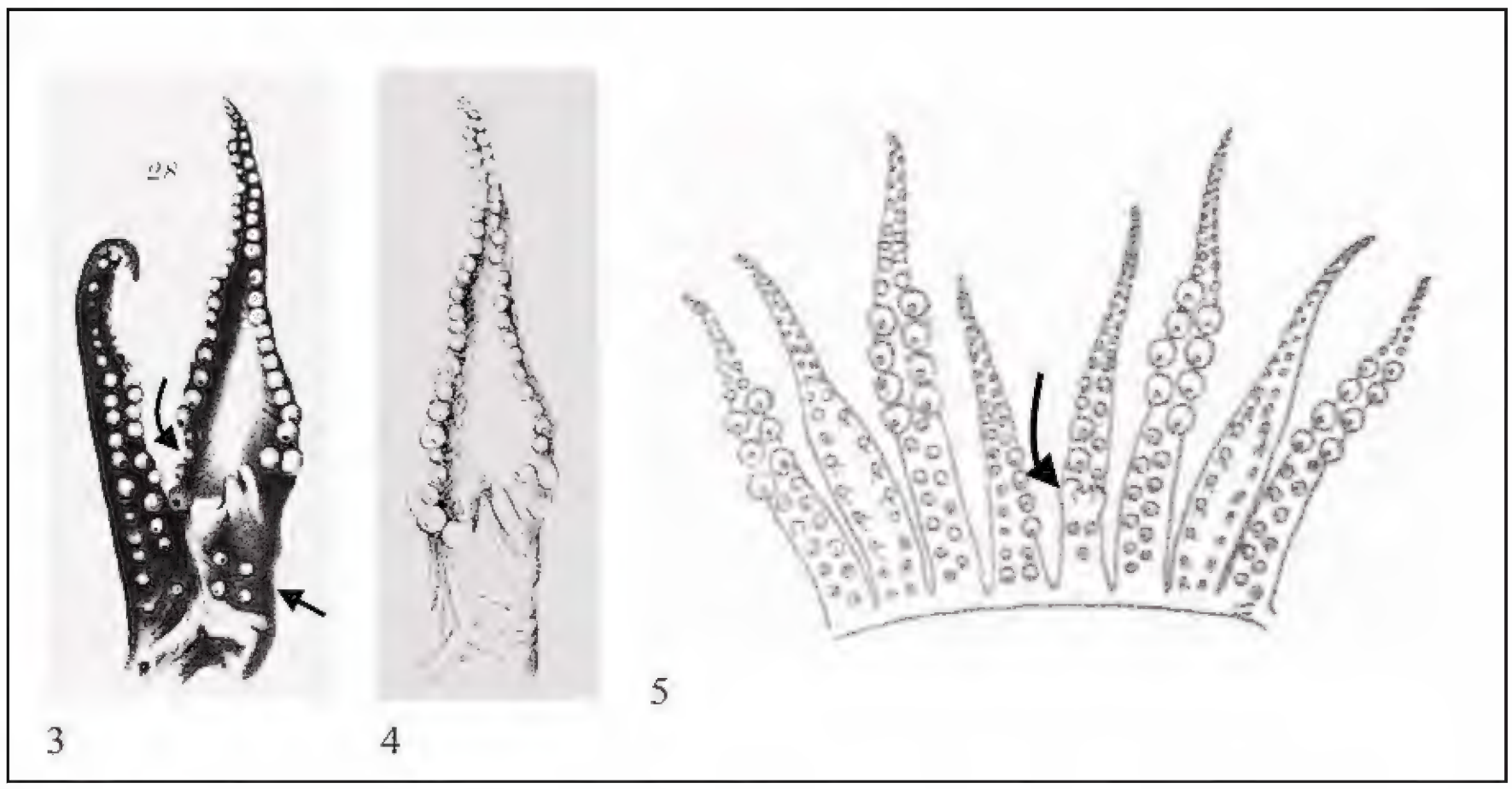

Figure 3. Drawing of the dorsal arms of purported *Sepiola rondeletii* from Jatta (1896: pl. 14 fig. 28). In fact, the depicted hectocotylus (arm on the right) is typically *Sepietta oweniana*'s according to the four basal suckers (straight arrow) (three in *S. rondeletii*) and the distribution of the enlarged suckers in two groups parted by few smaller suckers (curved arrow) in the dorsal row; the copulatory apparatus is not very indicative of any specific taxon. Compare Jatta's figure to Naef (1912b: fig. 1) (Fig. 6 of present paper), where the actual *S. rondeletii* and *S. oweniana* are labelled c and e, respectively.

Figure 4. Drawing of the hectocotylus of purported *Sepiola rondeletii* from Joubin (1902: fig. 2). This is a copy of a part of Jatta's figure (1896: pl. 14 fig. 28) (see Fig. 3 of present paper) and therefore depicts indeed the hectocotylus of *Sepietta oweniana*.

Figure 5. Arm crown of "*Sepiola rondeletii*" depicted by Pfeffer (1908: fig. 56). Naef (1923) stated that this belongs to *Sepiola intermedia*, according to the distribution of enlarged suckers and despite the fact that the copulatory apparatus (arrow) was inadequately delineated.

the Tyrrhenian Sea by the present author was unsuccessful. A further negative evidence is the list by Giordano & Carbonara (1999) which did not report this species among the cephalopods collected in 13 extensive bottom trawl surveys in that sea. In my opinion Würtz et al.'s (1995) record is not be taken into consideration. It was also doubted by Reid & Jereb (2005) by a question mark in the relevant distribution map.

A Mediterranean finding of *Heteroteuthis atlantis* was reported in a note of the paper describing the new species (Voss, 1955). Though this binomen was placed into synonymy with *Heteroteuthis dispar* by Nesis (1987), *Heteroteuthis atlantis* was subsequently cited, doubtfully yet, among the Mediterranean Sepiolidae (e.g. Bello, 1995). Nesis' (1987) opinion is shared by MolluscaBase (2018). Reid & Jereb (2005) state that until the taxonomy of the genus is studied, the validity of this heteroteuthine remains questionable.

Recent sepiolid Mediterranean entrance

Stoloteuthis leucoptera is the only extra-Mediterranean sepiolid that entered this basin in comparatively recent times. It is an amphi-Atlantic species (Reid & Jereb, 2005) that was first recorded in the Ligurian Sea thanks to three specimens collected there in 1988 (Orsi Relini & Massi, 1991) and afterwards was found in other western Mediterranean districts (Volpi et al., 1995; Würtz et al., 1995; Sánchez et al., 1998; Cuccu et al., 2010; Quetglas et al., 2013). The report by Quetglas et al. (2013), who netted 25 specimens mostly in the Alboran Sea, is particularly interesting because they started to capture this sepiolid only in 2001, while no one had been caught in previous surveys, from 1994 to 2010. In my opinion, the many Mediterranean occurrences of this cephalopod from the late '80s on, coupled with the lack of previous records from areas well surveyed in the past, soundly support the hypothesis that *Sepiola leucoptera* naturally entered the Mediterranean Sea from the Atlantic Ocean through the Straits of Gibraltar, extended its range within the western basin and succeeded in generating a self-sustained population (Battaglia et al., 2011). As for the Alboran Sea, the reported presence of *Sepiola leucoptera* since 2001 only (Quetglas et al., 2013) may be either due to a further immigration bout or to the westward expansion of the recent Mediterranean population. Lastly, heteroteuthines are known to lay their eggs on the sea floor (Nesis, 1993), hence the crossing of the Gibraltar Strait for sure involved juveniles and/or adults rather than early juveniles of this pelagic species.

The *Sepiola leucoptera* case makes a good example of the way the Mediterranean Sea has become naturally populated by Atlantic species after its re-connection to the ocean following the Messinian salinity crisis till the present.

Rare species

Three *Sepiola* species can be regarded as very rare, since each of them was only found in a very limited site, which coincides with its type locality. They are *Sepiola aurantiaca*, *Sepiola bursadhaesa* and *Sepiola boletzkyi*. The rarity of the latter two species may be just apparent because they were only recently described, hence they might have been overlooked if found in other district samples; in this respect, let us mention once more that members of Sepiolinae are the most difficult to identify species among the Mediterranean cephalopods especially when sexually immature.

As for *Sepiola aurantiaca*, the only known specimens were collected about one century ago exclusively in the spot called Amontatura, in the Gulf of Naples (Jatta, 1896). Until a few years ago, this sepioline had been reported in the eastern Atlantic Ocean as well, but Goud & de Heij (2012) showed that the Atlantic specimens belong indeed to *Sepiola pfefferi* Grimpe, 1921, a sister species of *Sepiola aurantiaca*. The Mediterranean rarity of the latter sepioline is quite puzzling especially when one takes into account that the Gulf of Naples is possibly the best explored place in the Mediterranean Sea thanks to the workers at the Stazione Zoologica, including Giuseppe Jatta, the species discoverer, and Adolf Naef, the Sepiolidae reviser, both of them keen cephalopod collectors.

Among the comparatively rare species, one may mention *Sepiola steenstrupiana*. In fact, this sepioline has been found in all Mediterranean districts (Mangold & Boletzky, 1987; Reid & Jereb, 2005). Its deceptive rarity depends on the fact that it lives in coastal areas only, at very shallow depths which are poorly explored with the customary trawl nets. Moreover, strangely enough, *Sepiola steenstrupi-*

ana has been reported from the Red Sea (Adam, 1973) and the western Indian Ocean (coasts of Somalia) (Rocha et al., 1998). This might be a rare case of counter-Lessepsian migration, from the Mediterranean to the Red Sea.

GENERAL REMARKS

In summary, updates for the Mediterranean Sepiolidae with respect to an earlier review of the Mediterranean teuthofauna (Bello, 2003) consist in: (a) the addition of the newly described species *Sepiola bursadhaesa* and *Sepiola boletzkyi*, one century after the mentioned discoveries by Naef (1912a, 1912b, 1912c; 1916); (b) evidence that *Sepiola aurantiaca* is endemic to this sea; (c) disclosure that *Sepiola rondeletii* is not widely distributed in the NE Atlantic Ocean but just close to the Gibraltar Strait, hence it is a quasi-endemic species; (d) evidence that *Sepiola ligulata* lives also in the NE Atlantic Ocean (it was deemed quasi-endemic by Bello, 2003; see also Reid & Jereb, 2005); (e) confirmation that *Stoloteuthis leucoptera* has established a viable population in the western basin. As for the Atlantic sepiolid diversity, in addition to the just conveyed news on *Sepiola rondeletii* and *Sepiola ligulata*: (f) a new species was described, namely *Sepiola tridens;* (g) *Sepiola pfefferi* was found to be the NE Atlantic sibling of the Mediterranean *Sepiola aurantiaca*.

As stated in Introduction, Sepiolidae is the most speciose cephalopod family in the Mediterranean (17 species) as well as in the NE Atlantic district (16 species); the overall NE Atlantic-Mediterranean

Figure 6. The hectocotylus diversity in many sepioline species (after Naef, 1912b: fig. 1). The drawings show the pair of dorsal arms - the hectocotylus is that on the right side – of *Sepiola steenstrupiana*, *S. robusta*, *S. rondeletii*, *S. aurantiaca*, *Sepietta oweniana*, *S. atlantica*, *S. ligulata*, *S. intermedia*, and *Rondeletiola minor* (left to right, starting from upper row).

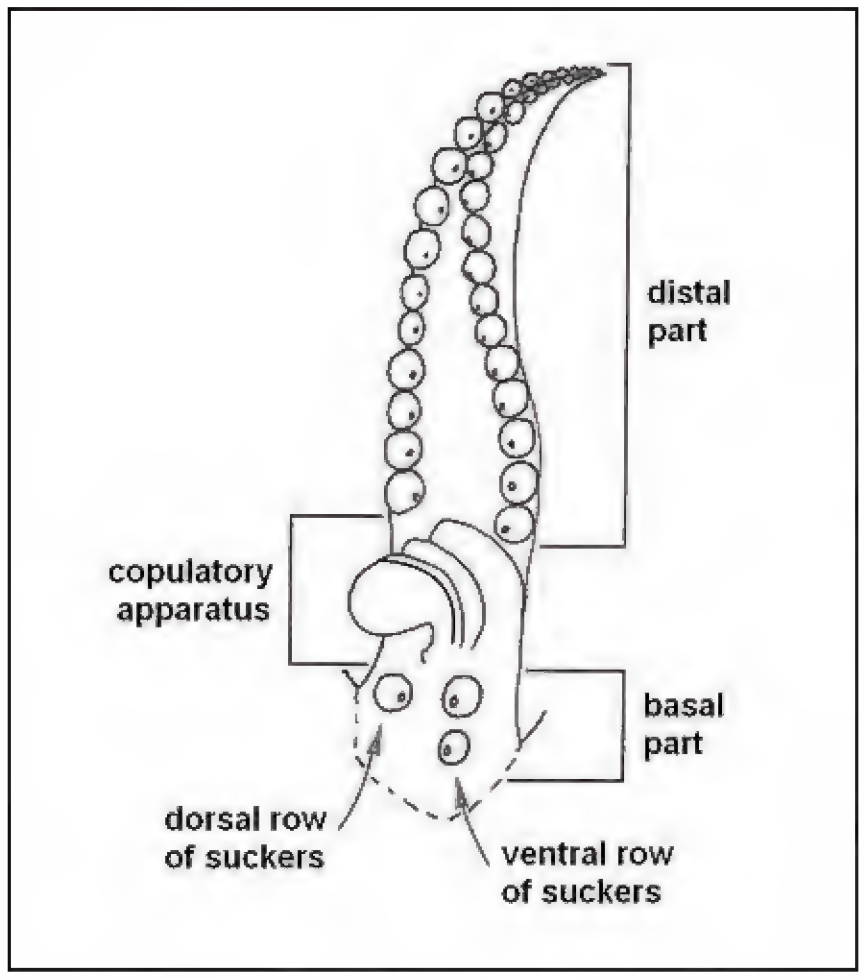

Figure 7. The hectocotylus basic type of the *Sepiola atlantica* group (sensu Naef, 1923). The copulatory apparatus is formed by four suckerless, elongate stalks fused with each other throughout their length into a structure apically directed and curled inwards; in some species the two dorsal suckers are not readily perceivable because of their strict fusion into a single tubercle.

sepiolid diversity includes 22 specific entities, 12 of which are shared by both districts (Table 1). The most speciose subfamily is Sepiolinae, which amounts to 13 specific entities in the Mediterranean, 10 in the NE Atlantic Ocean and overall 16, seven of which are found in both districts; in other words, this subfamily includes a fairly high fraction of endemic and quasi-endemic species: 69% in the Mediterranean Sea and 30% in the NE Atlantic. On the contrary, the few Mediterranean species belonging to Rossiinae and Heteroteuthinae live also in the North Atlantic Ocean; the only two endemic species in the latter district, namely *Rossia palpebrosa* and *Rossia moelleri*, live in high latitudes, well north of the Strait of Gibraltar.

The NE Atlantic-Mediterranean Sepiolinae form a fairly compact clade, where *Sepietta* and *Rondeletiola* are sister genera, that is synthetically (*Sepiola*, *Sepietta, Rondeletiola*)) (Bello, 1998). The males in this clade are characterized by a peculiar hectocotylus - the male left dorsal arm modified into a copulatory organ - where the copulatory apparatus is made of four modified suckers, two ventral and two dorsal (additional modified dorsal suckers may occur in some species) (Naef, 1923; Bello, 1995); the modification consists in the loss of the sucker proper and lengthening and/or widening of the stalk (Fig. 6) (Naef, 1923; Bello, 1995). The females bear a unique feature, that is an apomorphic character: the bursa copulatrix (Fig. 2).

A closer look at Sepiolinae reveals a fairly high number of twin- and sister-species (the former species displaying a closer affinity with each other than the latter) (Table 2). The majority of the *Sepiola* species, both Mediterranean and Atlantic, are gathered in the "*atlantica* group" sensu Naef (1923) because of their similarity (see also Bello, 2013) (Table 2). This group is characterized by the lengthening, fusion throughout their length, and inwards curling of the four modified sucker stalks of the copulatory apparatus (Fig. 7) (Naef, 1923; Bello, 2013). All of them are either Mediterranean endemics/quasi-endemics or NE Atlantic endemics, seven and two species respectively. Outside the "*atlantica* group", *Sepiola aurantiaca* and *Sepiola pfefferi* are twins and are endemic of the Mediterranean and the Atlantic Ocean respectively. The genus *Sepietta* contains fewer specific entities: a Mediterranean quasi-endemic, i.e. *Sepiola obscura*, and two widely distributed twin species, i.e. *Sepiola oweniana* and *Sepiola neglecta.*

GENESIS OF THE MEDITERRANEAN SEPIOLIDAE DIVERSITY

The Mediterranean Sea is generally deemed a sub-region of the Atlantic Ocean because its biodiversity originated with the Zanclean flood (5.3 MYA) following the Messinian salinity crisis (Blanc, 2002), which allowed the colonization by Atlantic organisms. The latter phenomenon has gone on since and is still at work; as for cephalopods, see for instance the above reported recent entrance and establishment of *S. leucoptera* in the western Mediterranean basin. One must point out that not all species entering the Mediterranean succeed in establishing a viable population here because the environmental conditions may be neither suitable for their survival nor for their reproduction (Bouchet & Taviani, 1992).

GENERA	SPECIES	NE ATLANTIC	MEDITERRANEAN	NOTES
Sepiola (*atlantica* group)	*affinis*		E	1
	atlantica	E		2
	boletzkyi		E	
	bursadhaesa		E	
	intermedia	(+)	QE	
	robusta		E	
	rondeletii	(+)	QE	
	steenstrupiana		E	
	tridens	E		2
Sepiola (others)	*aurantiaca*		E	3
	ligulata	+	+	
	pfefferi	E		3
Rondeletiola	*minor*	+	+	
Sepietta	*neglecta*	+	+	4
	obscura	(+)	QE	
	oweniana	+	+	4
Heteroteuthis	*dispar*	+	+	
Stoloteuthis	*leucoptera*	+	+	5
Rossia	*macrosoma*	+	+	
	moelleri	E		
	palpebrosa	E		
Neorossia	*caroli*	+	+	

Table 1. Composition of the north-eastern Atlantic and Mediterranean sepiolid-faunas. E: endemic species; QE: quasi-endemic species (see text); +: occurring in large part of district; (+): occurring close to Gibraltar Strait. NOTES: (1) The species of *Sepiola* in the "*atlantica* group" are phylogenetically closely connected with each other, hence they are sister species (Naef, 1923; Bello, 2013); (2) *atlantica* and *tridens* are twin species (de Heij & Goud, 2010); (3) *pfefferi* and *aurantiaca* are twin species (Goud & de Heij, 2012); (4) *oweniana* and *neglecta* are sibling species (Bello, 1998); (5) non-indigenous species recently established in the western Mediterranean (Battaglia et al., 2011).

MEDITERRANEAN SEA	NE ATLANTIC OCEAN
Sepiola aurantiaca	*Sepiola pfefferi*
Sepiola atlantica group (*affinis, boletzkyi, bursadhaesa, intermedia, robusta, rondeletii, steenstrupiana*)	*Sepiola atlantica* group (*atlantica, tridens*)

Table 2. Closely related NE Atlantic-Mediterranean pairs of *Sepiola* species.

Incidentally, additional, albeit unnatural, comparatively recent sources for the Mediterranean colonization are the opening of the Suez Canal, on 17 November 1869 and living creatures carrying in ship ballast water. In consequence of the former, some 500 Red Sea organisms have crossed the canal to reach the Levant Sea and, in several instances, spread out across most Mediterranean waters (Galil et al., 2017); they include some cephalopods too, e.g. *Sepioteuthis lessoniana* (Teuthida Loliginidae) (Salman, 2002). The latter source, i.e. ballast water, was invoked by Orsi Relini (2009) to explain the Mediterranean occurrences of the Indo-Pacific *Tremoctopus gracilis* (Octopoda Tremoctopodidae).

The natural colonization of the Mediterranean, a medium-latitude sea, by Atlantic organisms was heavily shaped by the sequence of Pleistocene glaciations and interglaciations that brought into the Mediterranean cold and warm elements respectively (Taviani, 2003). This phenomenon is typified by the rossiines: *Rossia macrosoma* and *Neorossia caroli* are respectively of Mauretanic (i.e. warm) and Lusitanic (i.e. cold) affinity (classification according to Ekman,1953). Parenthetically, Bello (2003) suggested that in a pair of akin species the cold-water one inhabits deeper layers than the warm-water one; this supposition is backed up by the distributions of the above-mentioned pair of rossiines.

The above paragraph implies that the two species of Mediterranean Rossiinae came from the near Atlantic Ocean. The same is true for the two species of Mediterranean Heteroteuthinae, whose entrance was further favoured by their mode of life, pelagic for *Heteroteuthis* dispar and bentho-pelagic for *Stoloteuthis leucoptera* (Reid & Jereb, 2005). The only NE Atlantic endemic species belonging to these two subfamilies live at high latitudes and most probably never moved southward to the level of the Strait of Gibraltar: *Rossia palpebrosa* is strictly temperate and *Rossia moelleri* is arctic (Reid & Jereb, 2005).

In addition to the species coming from the Atlantic Ocean, many new entities originated in the Mediterranean by in situ speciation after its re-colonization by Atlantic organisms. This statement concerns all marine taxa and, as for cephalopods, is particularly true for Sepiolinae, which, differently from the co-familiar subfamilies, display a high degree of endemism especially in the genus *Sepiola*.

Endemic members of this genus are also found in the Atlantic Ocean, namely *Sepiola atlantica*, *Sepiola tridens* and *Sepiola pfefferi* (Table 1). Mediterranean *Sepiola* species have their twin or sister counterparts in the ocean (Table 2), with the only exception of *Sepiola ligulata*, which occurs in both districts. This peculiar distribution is robust evidence to corroborate the close affinity between Mediterranean and Atlantic sepiolines and to support the hypothesis that both the Atlantic and Mediterranean *Sepiola* species originated from Atlantic common ancestors - possibly one for *pfefferi-aurantiaca*, which are twin species (Goud & de Heij, 2012), and another one for the "*atlantica* group" - that entered the Mediterranean and produced separate populations, which evolved independently to give rise to the present-day species. Moreover, some of the species that evolved in the Mediterranean basin went back through the Strait of Gibraltar and spread in its Atlantic vicinities, which species I termed quasi-endemic (Bello, 2003), namely *Sepiola intermedia* and *Sepiola rondeletii*. In particular, the situation of the "*atlantica* group" is quite interesting. As reported above, the members of this group share a peculiar hectocotylus, different from all other sepiolines. The examination and comparison of the hectocotylus of the "*atlantica* group" members show that its variations are just different expressions of a common proto-hectocotylus (proto- with respect to this group, not to the whole Sepiolinae) (Fig. 7).

As for the other genera in Sepiolinae, the species of *Rondeletiola* and *Sepietta* are found in both districts, although *Sepiola obscura* is a Mediterranean quasi-endemic, as reported above.

Two features appear of paramount importance in characterizing high-dispersal sepiolines (i.e. occurring in both the Atlantic Ocean and the Mediterranean) vs. low-dispersal species (endemic or quasi-endemic of either district): relative egg size (sensu Boletzky, 1974 and 1977) and, to a lesser extent, habitat depth. As shown by Boletzky (1974 and 1977), the egg relative size is responsible for the hatchling mode of life: the larger it is the shorter the planktonic life, hence less wide the spreading. In fact, the *Sepiola* species - all of which, with one exception, are endemic or quasi-endemic of either district - reproduce by comparatively large eggs (Gabel-Deickert, 1995) and live

mostly in shallow waters. The exception, namely *Sepiola ligulata*, though reproducing by large eggs, has been collected as deep as 350 m. The other comparatively deep water *Sepiola* species, i.e. *intermedia* and *robusta*, are quasi-endemic. *Rondeletiola* and *Sepietta*, on the contrary, include comparatively small-egged, deep-living species, with the exception of *Sepiola obscura,* which is large-egged and lives in shallow waters (Gabel-Deickert, 1995). Accordingly *R. minor*, *S. owenia-ana*, and *S. neglecta* are widely spread in both the Atlantic Ocean and the Mediterranean Sea, whereas *S. obscura*, as stated above, is a Mediterranean quasi-endemic.

According to Bello (2003), the high rate speciation in Sepiolinae is due to the following features: small body size; short life cycle (less than a year); reduced number of eggs, hence reduced fecundity, with respect to other Mediterranean cephalopods (Gabel-Deickert, 1996); reproduction by large eggs, which give birth to benthic early juveniles (Boletzky, 1974 and 1977); nekto-benthic mode of life (Bello & Biagi, 1995); mostly living in shallow coastal waters; fairly diverse hectocotylus, in males, and bursa copulatrix, in females (Naef, 1923; Bello, 1995). All these characteristics greatly favour speciation. For instance, the comparatively low-dispersal capabilities of both early juveniles and adults in shallow water habitats facilitates the establishment of small, marginal populations where the process of speciation may occur more efficiently (Eldredge & Gould, 1972). In fact, a few Mediterranean *Sepiola* species seemingly have a restricted distribution, e.g. *Sepiola aurantiaca* and *Sepiola bursadhaesa*. Bello (2003) also stated that sepiolines living in littoral environments appear to fit fairly well the statement of Hutchinson (1959) in his Homage to Santa Rosalia: "*... small size, by permitting animals to become specialized to the conditions offered by small diversified elements of the environmental mosaic, clearly makes possible a degree of diversity quite unknown among groups of larger organisms.*" (see Bello, 2003, for further comments). Moreover, a role in speciation is most probably also played by the species-specific lock-and-key copulatory organs, i.e. the male hectocotylus and the female bursa copulatrix, which preventing hybridization among allied sympatric species reinforces reproduction isolation (Hutchinson, 1959). In recent times, the lock-and-key mechanisms have been subjected to criticism and are no longer believed to depend on natural selection exclusively but rather on a combination of natural and sexual selection (Masly, 2012; Brennan & Prum, 2015). Whatever the evolutionary driving forces behind this selection, we can at least safely state that the species-specific hectocotylus-bursa copulatrix pairs in the Sepiolinae are the result of co-evolutionary processes.

CONCLUSIONS

We presently have at our disposal a fairly satisfactory picture of the systematics and biogeography of Mediterranean Sepiolidae, but there is still much to learn.

First of all, it must be stressed that, sadly enough, the literature is still crowded with misidentifications of sepioline specimens, including those used in genetic research (Groenenberg et al., 2009), which hinders the understanding of both their phylogenetic relationships and distribution, geographical as well as bathymetrical. Therefore, a prudent approach is suggested to workers dealing with sepiolid identification; on this respect a fair solution is the deposit of voucher specimens in official collections.

For a worldwide view, the phylogenetic relationships of the Atlantic-Mediterranean Sepiolidae with co-familiar members from the other oceans need to be studied on the basis of both morphological and genetic accounts. In turn, the ocean-wide biogeography of the family should be analyzed. This way, further light would also be shed on the Mediterranean situation of Sepiolidae.

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Biodiversity Journal, 2019, 10 (4): 405–408 https://doi.org/10.31396/Biodiv.Jour.2019.10.4.405.408
MONOGRAPH

Massive beaching record along the sandy coast of Catania (E-Sicily) of the rare "mole crab" *Albunea carabus* (Linnaeus, 1758) (Decapoda Anomura Hippoidea)

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ABSTRACT After a winter storm, with sirocco wind, hundreds of specimens of the "mole crab" *Albunea carabus* (Linnaeus, 1758) (Decapoda Anomura Hippoidea) were beached along the sandy coast of "Playa" Catania (E-Sicily). Even though this species was previously recorded in some scattered localities of the Mediterranean, among which a couple were found in Sicily, the present record appears relevant on account of the massive finding of specimens, which is a quite rare event according to literature data. Considerations on the reasons of this wide beaching complete the present note.

KEY WORDS Mole crab; *Albunea carabus*; massive beached; river estuary; Catania, Mediterranean Sea.

Received 10.07.2018; accepted 12.09.2018; published online 20.12.2019
Proceedings of the 4th International Congress on Biodiversity "Man, Natural Habitats and Euro-Mediterranean Biodiversity", November 17th-19th, 2017 - Malta

INTRODUCTION

Amongst Crustacea, Decapoda, which include crayfish, crabs, lobsters, prawns, and shrimp are a highly diverse group since they are adapted to many different marine, freshwater and terrestrial environments. The superfamily Hippidea, commonly known as mole crabs, are Decapoda which includes three families. In the family Albuneidae the genus *Albunea* Weber, 1795 comprises 22 species (Boyko, 2002), of which *Albunea carabus* (Linnaeus, 1758) is present in the Mediterranean, from which it was described for the first time. In fact, the first description of *A. carabus* was performed by Linnaeus (1758) as *Cancer carabus* on specimens from Algiers. Some confusions suddenly happened on the morphological character of the rostrum when Linnaeus described it as formed by "*two parallel and movable teeth*", but Miers (1878) stated that he was describing the ocular peduncles. We have no type materials of this species and, according to Boyko (2002), no neotype designation is needed since this is the only albuneid present in east Atlantic and the Mediterranean. A complete diagnosis of this species is found in Boyko (2002), who cited it as the main representative species of the "*carabus*-group" of *Albunea*, which also comprises two other extant species, *A. danai* Boyko, 1999 and *A. bulla* Boyko, 2002, and two Miocenic species, *A. asymmetrica* (Muller, 1979) and *A. turritellacola* (Fraaije, van Bakel et Jagt, 2008).

Albunea carabus is widespread along the Eastern Atlantic coasts, in Western African regions,

from Benin to Ivory Coast, Cape Verde Islands included, and in Portugal, Algarve coast and Madeira Islands. In the Mediterranean it is scattered from eastern (Katagan & Cem, 2003) to western regions (see Pereira et al., 2008 for a comprehensive list of record's localities). Everywhere it is considered very rare, but, according to Boyko (2002), it could be more common on its favorite substrates, which are low sandy bottoms subjected to high hydrodynamism, where it borrows often in proximity of estuaries (Giacobbe & Spanò, 1996; Spanò et al., 1999; Boyko, 2002; Pereira et al., 2008).

Our material was washed ashore quite abundantly in E-Sicily, in an area north to Simeto, the longest river in the island, and near another minor creek. This finding offered us the possibility to document a massive beaching of this species in the Mediterranean and to create a parallelism with preceding data for the Atlantic Ocean.

MATERIAL AND METHODS

After a storm in February 2017, approximately two hundreds of specimens were found along a limited stretch of the southern and sandy beach of Catania ("Playa") which is extended up to 18 km in its whole extension. The inspected area (Fig. 1) is comprised between the southern jetty of the harbor ("Sciara Biscari" zone), where the small creek Acquicella flows into the sea, and 500 m south at the first free entrance to the beach from the main street. At the moment of the finding a slight wind of Sirocco was present and specimens were accumulated by the low waves along the sandy beach, where marine birds, seagulls and egrets, and stray dogs fed upon them. Only 40 entire specimens were collected (Figs. 2–8), 15 females and 25 males, ranging from 21.2 mm (male) to 40.2 mm (female); they were preserved in ethanol.

In addition to the mole crabs, other species of crabs were found, among which the Brachiurids *Portumnus latipes* (Pennant, 1777), *Portunus hastatus* (Linnaeus, 1767) and *Portunus segnis* (Forskål, 1775). A further note should be added on the terrestrial and freshwater material beached together with the "mole crabs". Numerous undetermined freshwater fishes, probably decomposed specimens of *Tinca tinca* (Linnaeus, 1758), frogs as *Pelophylax esculentus* (Linnaeus, 1758), lizards as *Chalcides ocellatus* (Forsskål, 1775), *Podarcis siculus Rafinesque*, 1810 and *Lacerta bilineata* Daudin, 1802, snakes as *Natrix natrix* Linnaeus, 1758) and mammals as *Rattus norvegicus* Berkenhout, 1769, were contemporaneously found among the very large amounts of residuals of the river cane *Phragmites australis* (Cav.) Trin. ex Steud., here often beached by the winter storms.

RESULTS

Albunea carabus (Figs. 3, 4) can be distinguished by the triangular ocular plates (Fig. 5), almost squared in *A. bulla*, and for the subquadrate ventral margin of the heel of the *pereopod II dactylus* (Fig. 6), rounded in the sister species *A. danai*. Females are characterized by a larger and more rounded in shape last segment of the telson (Fig. 7), instead of almost triangulate in males (Fig. 8). Though it appears to be a well distributed species, along the Mediterranean coasts *A. carabus* seem widely distributed, from Eastern to Western regions, but inside restricted environments and with very scarce materials collected (few specimens for each locality). Only the record of Piguet (1955) is known to report a massive beaching of this species for Algerian sandy coast in the same conditions. So, the present finding assumes a particular significance when compared to the others. First of all because our data confirm the environmental preferences known in literature for this almost elusive species: the sandy bottom (SFBC according to Pérès & Picard, 1964), almost brackish water and the high hydrodynamism. Moreover, the finding of numerous terrestrial and freshwater species of animals and plants suggests that a sudden river overflow occurred, which surprised all these organisms, killed them, producing in the marine ecosystems an exceptional wave of freshwater along the Simeto's surroundings, which caused the massive death of the mole crabs. This explains the large number of specimens collected in the same beach at the same time. Our data confirm therefore this remarkable event, only occasionally reported in literature: the freshwater runoff, in fact, is suggested by Piguet (1955) as possible cause of mortality of a large number of specimens in Algiers.

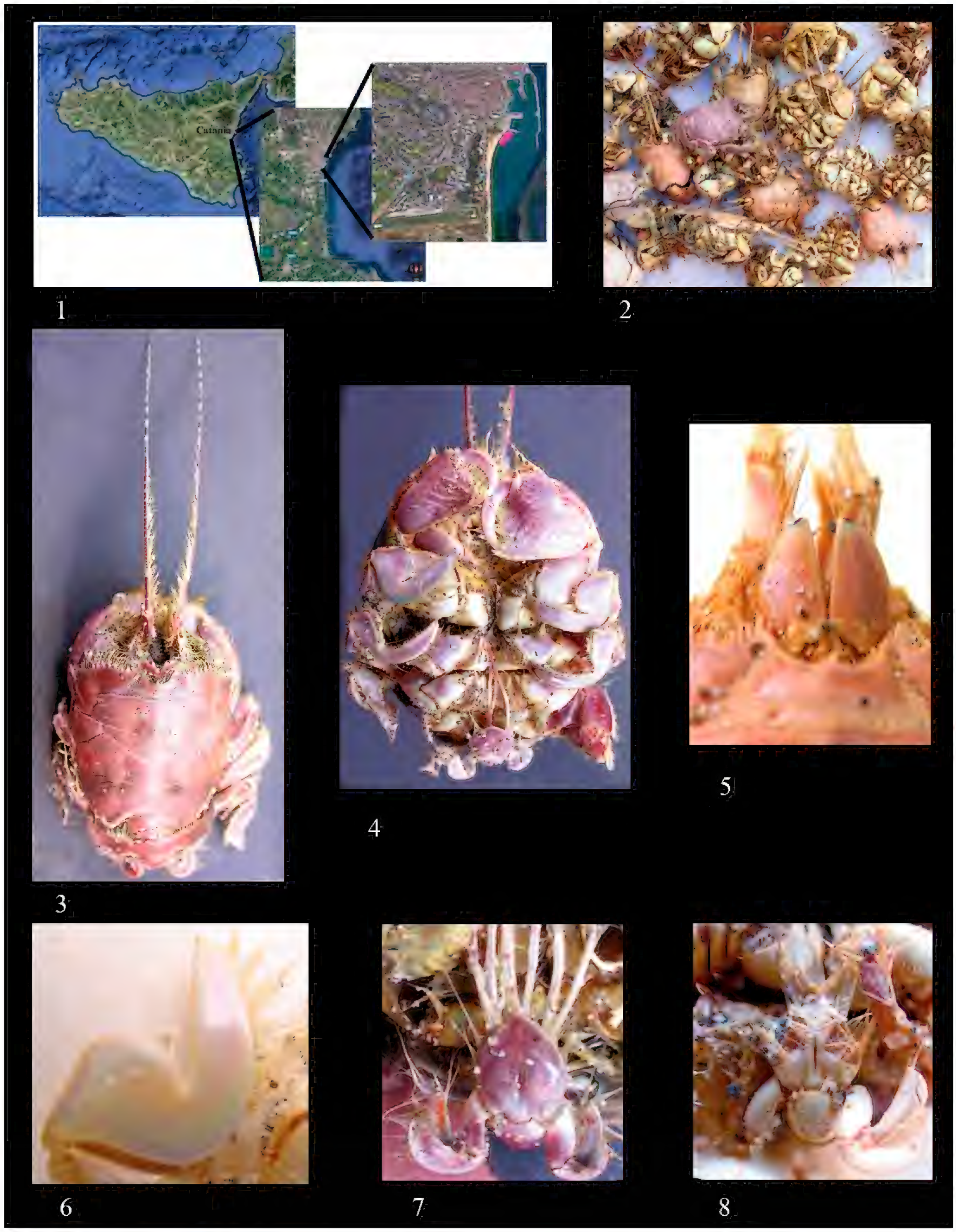

Figure 1. Map of the E-Sicily (Italy) with details of the harbor of Catania: pink colored area represents the stretch of the sandy beach where the collected material was found. Figures 2–8. *Albunea carabus* from Catania "Playa", E-Sicily. Fig. 2: *Albunea carabus* from Catania "Playa". Fig. 3: whole specimen (female) in dorsal view, h 39.4 mm. Fig. 4: the same specimen in ventral view. Fig. 5: detail of the ocular plates. Fig. 6: detail of the ventral margin of the heel of the pereopod II dactylus. Fig. 7: detail of the female telson. Fig. 8: detail of the male telson.

ACKNOWLEDGMENTS

We are grateful to prof. Carlo Froglia (CNR-IRBIM, Ancona, Italy) for interesting suggestions and literature assistance.

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Biodiversity Journal, 2019, 10 (4): 409–414 https://doi.org/10.31396/Biodiv.Jour.2019.10.4.409.414
MONOGRAPH

New taxonomical and biological observations on *Jujubinus seguenzae* Ghisotti et Melone, 1975 (Gastropoda Vetigastropoda Trochidae)

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ABSTRACT The study of numerous shells in the collection of the Authors and the finding of new material, among which a single living specimen, some kilometers South to the known geographical area of distribution, allowed us a better accurate morphological study of the shell, the protoconch shape and sculpture and the anatomy and color pattern of the external soft parts. Thus new data allowed to enlarge its geographical distribution and the current taxonomical status of the species. Comparisons with shell and soft parts of similar congeners are made. In particular, differences between *Jujubinus seguenzae* Ghisotti et Melone, 1975 and the main "morphs" of *J. striatus* (Linnaeus, 1758) (Gastropoda Vetigastropoda Trochidae) will be underlined and the possibility that this last taxon would be better re-defined on the basis of the original materials is discussed.

KEY WORDS *Jujubinus seguenzae* complex; Trochidae; Recent; Mediterranean Sea; living animal.

Received 10.07.2018; accepted 12.09.2018; published online 20.12.2019
Proceedings of the 4th International Congress on Biodiversity "Man, Natural Habitats and Euro-Mediterranean Biodiversity", November 17th-19th, 2017 - Malta

INTRODUCTION

The family Trochidae, top-snails or top-shells, have recently received a lot of attention, especially representatives of the genus *Jujubinus* Monterosato, 1884. A recently updated checklist for Trochidae listed 18 extant species of this genus present in the Mediterranean Sea. It is still unclear whether some of these species should be considered subspecies. Among these latter species we can consider *J. seguenzae* Ghisotti et Melone, 1975, known for the shallow waters of the Strait of Messina, although living specimens were never till now documented.

This species is currently considered a nomen dubium (WoRMS citing Oliverio, 2006 as a font). This is probably due to the lack of biological informations and its morphological resemblance with *J. striatus* (Linnaeus, 1758), to which Ghisotti & Melone (1975) connected it. No papers had never really discussed its taxonomical position, except for the paper of Cretella (1993), who proposed a good schematic re-description of the species, so considering it as valid.

The exam of photographs of the type material of *J. striatus* in the Linnean collection convinced us that it is constituted by more than one species, but no one attributable to *J. seguenzae*. It probably rep-

resents a complex of species, some of which were described as new in recent time, i.e. *J. curinii* Bogi et Campani, 2006, *J. trilloi* and *J. eleonorae* Smriglio, Di Giulio et Mariottini, 2014, *J. alboranensis* Smriglio, Mariottini et Oliverio, 2015, while others still continue to have an uncertain taxonomical position, i.e. *J. fraterculus* (Monterosato, 1879), considered as valid by Scuderi & Terlizzi (2012), or *J. striatus delpreteanus* Sulliotti, 1889, which was never formally collected and treated in recent time.

The aim of the present paper after new recent findings is to well characterise the taxonomical position of *J. seguenzae,* resolving the question whether it could be considered a good species. In addition, we trace again its distributional geographic area and we give some new morphological informations, among which the resemblance of the external soft parts, for which the features and colour of snout lappets and of the sense organs at the base of the pedal tentacles are here reported as taxonomically discriminant.

MATERIAL AND METHODS

Morphological studies were conducted on the dry materials of the "*J. striatus* complex" species of our own collections: complete data are reported under in the examined material. Specimens were counted and measured. Live collected specimens were obtained by brushing small stones covered by green algae in shallow waters and sampling on the leaf stratum the vagile fauna using the hand-towed net method by a 40x20 cm hand-towed net with a 0.5 mm mesh. Drawings of the external soft parts were made for comparisons to similar congeners. For the evaluation of the shape and the sculpture of the protoconch SEM photographs of the studied taxa were made.

ACRONYMS AND ABBREVIATIONS. AVC: Alberto Villari collection, Messina, Italy; DSC: Danilo Scuderi collection, Catania, Italy; sh/s.: empty shells; sp.: living collected specimen/s.

RESULTS

Systematics

Classis GASTROPODA Cuvier, 1795
Subclassis VETIGASTROPODA Salvini-Plawen, 1980

Figure 1. Map of the distribution of *Jujubinus seguenzae* in eastern Sicily: pink colored area represents the distribution of the species according to literature data, confirmed by material seen by the authors; yellow colored areas show the new findings out of the Strait of Messina.

Ordo TROCHIDA Rafinesque, 1815
SuperfamiliaTROCHOIDEA Rafinesque, 1815
Familia TROCHIDAE Rafinesque, 1815
Subfamilia TROCHINAE Rafinesque, 1815
Genus *Jujubinus* Monterosato, 1884

TYPE SPECIES. *Trochus matonii* Payraudeau, 1826 = *Jujubinus exasperatus* (Pennant, 1777) by subsequent designation.

Jujubinus seguenzae Ghisotti et Melone, 1975

EXAMINED MATERIAL. Italy. Ganzirri, Messina, N-E Sicily, 18 sh., shell grit collected at -2/4 m and beached (DSC). Riviera Paradiso, Messina, N-E Sicily, 20 sp., on floating algae of the genus *Ulva* (AVC). Riviera Paradiso, Messina, N-E Sicily, 35 sp., on the sea-grass *Posidonia oceanica* (AVC). San Raineri, "Degassifica" station, Messina, N-E Sicily, 4 sp., shell grit collected at -4/8 m (AVC). Furci Siculo, Messina N-E Sicily, 2 sp., on rocks with a thin algal film (AVC). Giardini Naxos, Messina, E Sicily, 1 sh., beached (DSC). Riposto, Catania, E Sicily, -6/20 m, 19 sh. collected by a 20 litres grab and 1 sp. brushing handily little stones covered by green algae (DSC).

Jujubinus depictus. Italy. Taormina, Messina, N-E Sicily, 23 sh. on rocks, -4/6 m (AVC). Ganzirri, Messina, N-E Sicily, 33 sh., shell grit collected at -2/4 m (AVC). San Giovanni Li Cuti/Cajto, Catania, E Sicily, 311 sh. and 25 sp., shell grit collected at -2/22 m and fishing nets (DSC).

Jujubinus striatus. Italy. Acitrezza, Catania, E Sicily, 22 sh., fishing nets, -10/20 m (AVC). Riviera Paradiso, Messina, N-E Sicily, 76 sh. on *Posidonia oceanica*, -4/8 m (AVC). Taormina, Messina, N-E Sicily, 43 sh. on *Posidonia oceanica*, -4/10 m (AVC). Brucoli, Siracusa, S-E Sicily, 38 sp., beached shell grit (DSC). Marzamemi, Siracusa, S-E Sicily, 18 sp., hand-towed net method on *Posidonia oceanica*, -4/6 m (DSC).

Jujubinus striatus delpreteanus. Italy. Lago Faro, Messina, N-E Sicily, 135 sh., shell grit collected at -1/2 m (AVC/DSC).

Jujubinus exasperatus. Italy. Terrauzza, Siracusa, S-E Sicily, 1 sp., hand-towed net method on *Posidonia oceanica,* 4/6 m (DSC).

Jujubinus tumidulus. Italy. Ganzirri, Messina, N-E Sicily, 41 sh., shell grit collected at -100/120 m by fishing nets (AVC/DSC).

DESCRIPTION. Shell. We refer to the good description of Cretella (1993) for the general shape, dimensions, colour and sculpture of the teleoconch, which is well characterized by a more conic shell in comparison to that of the other congeners. Further diagnostic characters on the teleoconch, protoconch and external soft parts and comparisons are here added.

Teleoconch: general shape is an almost high regular cone (Figs. 2-6), with up to 5.5 flat whorls, the first of which is characteristically dome-shaped. Protoconch and initial teleoconch whorls are of the same colour of the rest of the shell. Whorls are sculptured by 5-8 almost flat and smooth spiral ridges, larger than interspaces, which sometimes could be divided each in two smaller cordlets; dense prosocline growth lines between interspaces and over the spirals. The two adsutural and absutural are well marked, closer and sometimes incised by growth lines, which could form not well marked granulations (Fig. 7).

The base is slightly convex to almost flat, without umbilicus. Peristome squared, only slightly rounded in some specimens at the lower external corner. The columellar wall is straight until 1/3 down of its length, where a not marked columellar fold is present. Colour gray-yellowish, with white and brownish alternated and opisthocline large flames, which in the spiral ridges appear as small alternated dots. Dark-gray and yellowish specimens with small alternated dots are not uncommon (Fig. 5).

Protoconch constituted by 1 flat whorl, almost at the same plan of the tele-whorls (Figs. 8, 9). Sculpture is formed by little but numerous and scattered granules of irregular shape, which merge in two faint spiral treads, one in the middle and one uppermost.

External soft parts (Fig. 23): general feature as in *Jujubinus* species. Foot well developed, granulose on the surface, cream in ground colour, with brown small dashes and big spots arranged in two main rows and one of smaller spots, alternated to white one, along the epipodial ridge, which bears a wide and pointed denticulated margin; numerous expansions at the lower margin; white tail of the foot; two long and tapered cephalic tentacles with dentate sides and thin dark little lines and small white dots along the entire length; three couples of epipodial tentacles similar in shape and colour to

the cephalics, with white knob-shaped sense organ at the base (Fig. 25); snout long, dark-brown, with a whitish line before the tentaculated margin of the mouth and two gray lappets with white dots; of the same dark colour are washed the uppermost side of the eyes, the forehead and the neck, where a "V" shaped draw is painted; both neck lobes not digitated, gray with white stains; the glandular area under operculum forms a horseshoe draw (Fig. 24).

DISTRIBUTION. The species was originally reported as endemic to the Strait of Messina, as well as in the following papers and single citations. Materials here studied allowed us to enlarge this area to other two more Southern localities: Giardini Naxos, in the same territory of Messina, and Riposto, which is comprised in the northern territory of Catania, 60 km South to the type locality (Fig. 1).

DISCUSSION

The exam of the diagnostic morphological characters of *J. seguenzae* do not leave any doubt in considering it as a good separated species from the other congeners, especially from those of the *J. striatus* group, which are morphologically related.

Notwithstanding the doubts expressed by Cretella (1993) on the correct first institution of the taxon ("… *Ghisotti & Melone …, who designate it, however, as a form connectible to* J. striatus"), we think that the AA., although briefly, wanted to describe a real new species, only similar to *J. striatus*. Numerous new species of the "*J. striatus* group" were described in recent times, but are morphologically different from *J. seguenzae* and seem confined to other restricted geographical areas. Moreover, a possible relation of *J. seguenzae* with *J. striatus delpreteanus*, a controversial taxon typical of the Faro lake, northern to Messina (Fig. 14), whose taxonomical position is still debated, is excluded after comparisons to topotypical specimens. This latter, in fact, is characterized by a shell almost thin with inflated whorls and sculpture of numerous and almost smooth spiral chords alternated with rough interspaces.

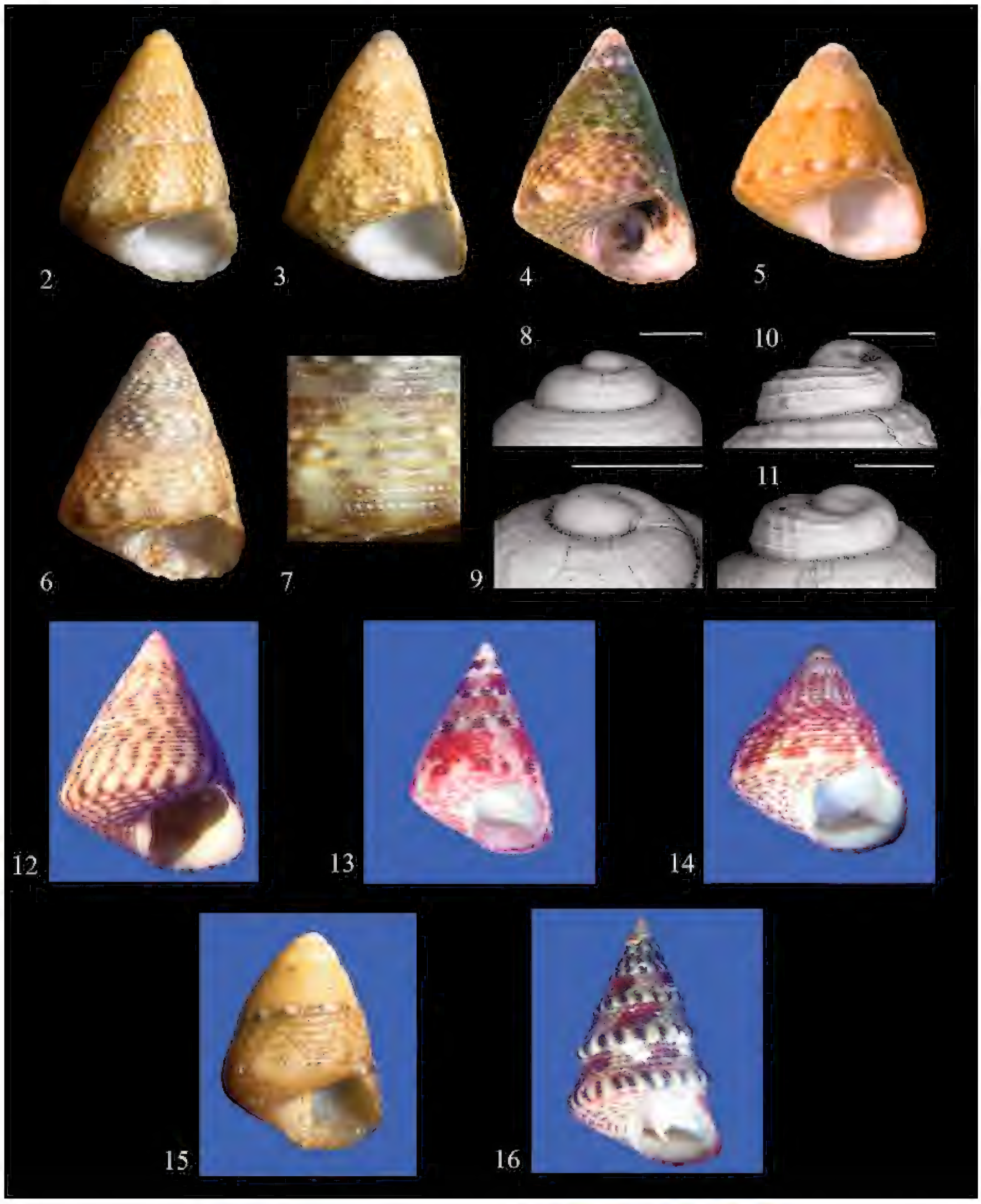
Instead, *J. seguenzae* shows more affinities to what is here considered the true *J. striatus* (Fig. 12), on account of original pictures and photographs of the type material (The Linnean Collections, *Trochus striatus*), and to what is here called *J. depictus* (Fig. 13), whose taxonomical definition needs further and appropriate studies, but which we consider different from the nominal species on account of differences observed in protoconch shape and sculpture (Fig. 10), external soft parts (Figs. 17–19) and ecological habits, being the former typical of sea-grass beds and the latter of rocky bottom's algae. In fact, besides a different pattern of the foot and stain drawings on the head, *J. striatus* (Figs. 20–22) and *J. depictus* (Fig. 17) show different form of the sensory organs at the base of the epipodial tentacles and colour and form of the mouth lappets. But, compared to them, *J. seguenzae* has a more cyrtoconoid shape, thick abapical cords with tubercles (Fig. 7), different colour pattern of the shell and of the external soft parts and a different protoconch (Figs. 8-9). Another debated taxon of the same complex, *J. fraterculus* (see Scuderi & Terlizzi, 2012 for instance), has an almost greenish animal (Cecalupo et al., 2008 and pers. obs.) and a shell sculpture and general outline which make it similar to the two preceding species. For the same reasons above underlined, it is well distinguishable from *J. seguenzae*.

Another morphologically similar species, also present in the same geographical area, is *J. tumidulus* (Aradas, 1846), which have a more rounded and stouter outline of the shell, sculptured by granulated instead of smooth medial spiral ridges (Fig. 15).

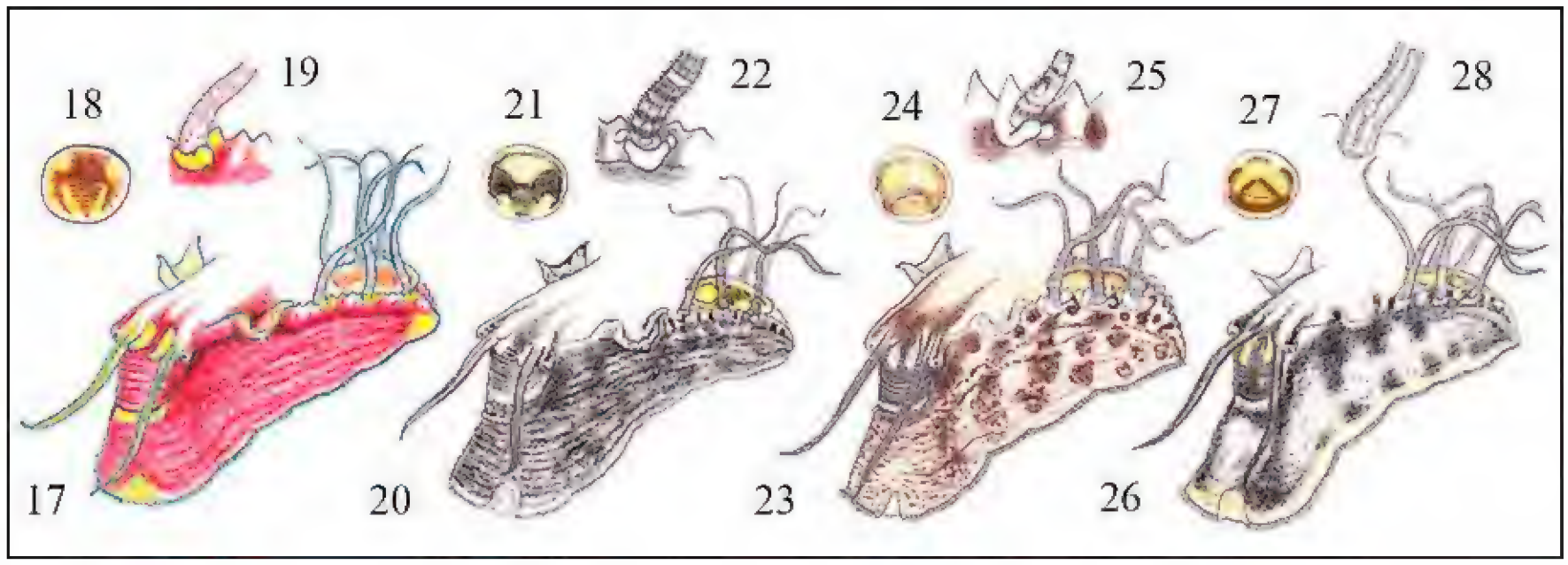
The protoconch with the first tele-whorl is almost flat in *J. seguenzae* (height/diameter ratio: 0.46) (Fig. 8) and not slightly protruding as in *J. striatus* (height/diameter ratio: 0.5) (Fig. 11) and highly protruding as in *J. depictus* (height/diameter ratio: 0.6) (Fig. 10). Moreover *J. seguenzae* has only one flattened protoconch whorl, *J. striatus* 1.3 inclined whorls and *J. depictus* 1.2 highly inclined whorls. The protoconch sculpture is more faint in *J. seguenzae* and in *J. striatus* than in *J. depictus*.

A similar protoconch, together with the peculiar hydrodynamism conditions of the Strait of Messina and the marked preferences for a specific environment - SGCF (coarse sands and fine gravels under the influence of bottom currents) according to Pérès & Picard (1964) - suggest for *J. seguenzae* a lower dispersal capability, notwithstanding the geographical distributional area is here enlarged to more southern areas, characterized however by the same biocenotic assemblage.

J. seguenzae lives on marine sea-grass and green algae. The finding of some specimens on a



Figures 2–9. *Jujubinus seguenzae* from Italy. Fig. 2: S. Raineri, "Degassifica" station, Messina, h 5.4 mm. Fig. 3: Ganzirri, Messina, h 5 mm. Fig. 4: Riposto, Catania, live collected, h 6.1 mm. Fig. 5: idem, h 2.4 mm. Fig. 6: Furci Siculo, Messina, h 4.5 mm. Fig. 7: idem, detail of the shell sculpture. Fig. 8: S.E.M. photograph of the protoconch, same data of the specimen in figure 4 (scale bar 200 µm). Fig. 9: S.E.M. photograph of the protoconch, detail of the nucleus, same data of the specimen in figure 4 (scale bar 200 µm). Fig. 10: *J. depictus*, Catania, S.E.M. photograph of the protoconch (scale bar 200 µm). Fig. 11: *J. striatus*, Brucoli, Siracusa, S.E.M. photograph of the protoconch (scale bar 200 µm). Fig. 12: *J. striatus*, Brucoli, Siracusa, h 10 mm. Fig. 13: *J. depictus*, Catania, h 5.1 mm. Fig 14: *J. striatus delpreteanus*, Lago Faro, Messina, h 5.9 mm. Fig.15: *J. tumidulus*, Ganzirri, Messina, h 3.4 mm. Fig. 16: *J. exasperatus*, Terrauzza, Siracusa, h 8.0 mm.



Figures 17–19: *Jujubinus depictus,* San Giovanni Li Cuti Catania, Italy. Fig. 17: drawing of the external soft parts, San Giovanni Li Cuti Catania, Italy. Fig. 18: detail of the glandular area under operculum; Fig. 19: detail of sense organs of epipodial tentacles. Figures 20–22: *J. striatus,* Marzamemi, Siracusa, Italy. Fig. 20: drawing of the external soft parts. Fig. 21: detail of the glandular area under operculum; Fig. 22: detail of sense organs of epipodial tentacles. Figures 23–25: *J. seguenzae*, drawing of the external soft parts of the specimen in figure 4; Fig. 24: detail of the glandular area under operculum; Fig. 25: detail of sense organs of epipodial tentacles. Figure 26: *J. exasperatus*, drawing of the external soft parts of the specimen in figure 16; Fig. 27: detail of the glandular area under operculum; Fig. 28: detail of sense organs of epipodial tentacles.

floating leaf of *Ulva* sp. suggests the possibility of the species to disperse in surrounding geographical sites taking advantage of these large-leaf algae transported by the sea currents. It is remarkable, however, that the above described environment is not present far Southern to Riposto, being the characteristics of the bottom influenced by both the lava stones and the sand of the Southern part of the gulf of Catania.

ACKNOWLEDGEMENTS

We are grateful to prof. Marco Curini-Galletti (Università degli Studi di Sassari, Italy) for his kind availability for literature assistance and interesting suggestions.

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Biodiversity Journal, 2019, 10 (4): 415–426 https://doi.org/10.31396/Biodiv.Jour.2019.10.4.415.426
MONOGRAPH

Are *Pinctada radiata* (Leach, 1814) and *Pinctada fucata* (Gould, 1850) (Bivalvia Pteriidae) only synonyms or really different species? The case of some Mediterranean populations

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ABSTRACT

The earliest reported alien species that entered the Mediterranean after only nine years from the inauguration of the Suez Canal was "*Meleagrina*" sp., which was subsequently identified as the Gulf pearl-oyster, *Pinctada radiata* (Leach, 1814) (Bivalvia Pteriidae). Thereafter, an increasing series of records of this species followed. In fact, nowadays it can be considered a well-established species throughout the Mediterranean basin. Since the Red Sea isthmus was considered to be the only natural way of migration, nobody has ever doubted about the name to be assigned to the species, *P. radiata*, since this was the only *Pinctada* Röding, 1798 cited in literature for the Mediterranean Sea. Taxonomy of *Pinctada* is complicated since it lacks precise constant morphological characteristics to distinguish one species from the others. Thus, distribution and specimens location are particularly important since different species mostly live in different geographical areas. Some researchers also used a molecular phylogenetic approach, but the results were discordant. This taxonomic conundrum was re-examined this time applying morphological taxonomy. Increasing vessel traffic and records of vast amounts of *Pinctada* specimens with morphologically different shells led us to formulate the hypothesis that a separate *Pinctada* population of different geographical provenance could be present. Specimens were grouped according to the site of collection in the Mediterranean basin. Results from these morphological studies confirmed that, according to us, there were two distinct species, *P. radiata* and *P. fucata* (Gould, 1850). Morphological comments and interpretations on the taxonomical status of both species together with auto-ecological notes and a literature review of the molecular phylogenetic studies conducted will be here presented.

KEY WORDS *Pinctada imbricata* complex; Pteriidae; pearl oyster; Recent; Mediterranean Sea.

Received 10.07.2018; accepted 12.09.2018; published online 20.12.2019
Proceedings of the 4th International Congress on Biodiversity "Man, Natural Habitats and Euro-Mediterranean Biodiversity", November 17th-19th, 2017 - Malta

INTRODUCTION

Just only nine years after the inauguration of the Suez Canal, Monterosato (1878) reported for the first time "*Meleagrina*" sp. for the Mediterranean Egypt (Alexandria), where it was so abundant that it was sold in the local fish markets.

He admitted not to know whether it was a local

still unknown species or the earliest reported lessepsian species, as it was. Subsequently the species was doubtfully described as new as *Meleagrina savignyi* Monterosato, 1884 (Bivalvia Pteriidae), later identified as *Pinctada radiata* (Leach, 1814) (Parenzan, 1961; Bombace, 1967; Paccagnella, 1967; Spada, 1969).

Since modern times, this latter name has never been questioned and nobody has ever doubted if under this name more than one species could be hidden.

Taxonomy of the species of *Pinctada* Röding, 1798 is complicated because of the extreme variability of species in this genus and seems far to be clearly defined. In fact *P. radiata* has been considered as conspecific with *P. fucata* (Gould, 1850) (see Hynd, 1955) and, later on, as mere synonyms (Ranson, 1961) ("alternate representation" in WoRMS).

Some important factors, like wide geographical distribution and anthropic contribution to hybridization, are reported by Temkin (2010) as affecting the extreme polymorphism of species.

As a consequence, a molecular phylogenetic approach was used by some researchers but the results were still discordant: see for instances Temkin (2010) and Cunha et al. (2011).

For this reasons, the former Author stated the lacking to date of precise constant morphological characters, in particular to discriminate species of the so called "*P. radiata*" group, for which three different clines have been distinguished and taxonomically related by him to geographical sub-species: *P. imbricata imbricata* Röding, 1798 in the western Atlantic areas, *P. imbricata radiata* in the eastern Indian Ocean and the Red Sea areas and *P. imbricata fucata* in the Indo-Pacific areas.

As the modes of introduction of this pearl oyster in the Mediterranean Sea, more than one have been described in the literature: intentional introduction by mariculture; shipping, which is considered to be the most likely introduction vector of non-indigenous species (Zibrowius, 1992) and even by migrant trips of the sea turtle *Caretta caretta* (Linnaeus, 1758) (Oliverio et al., 1992).

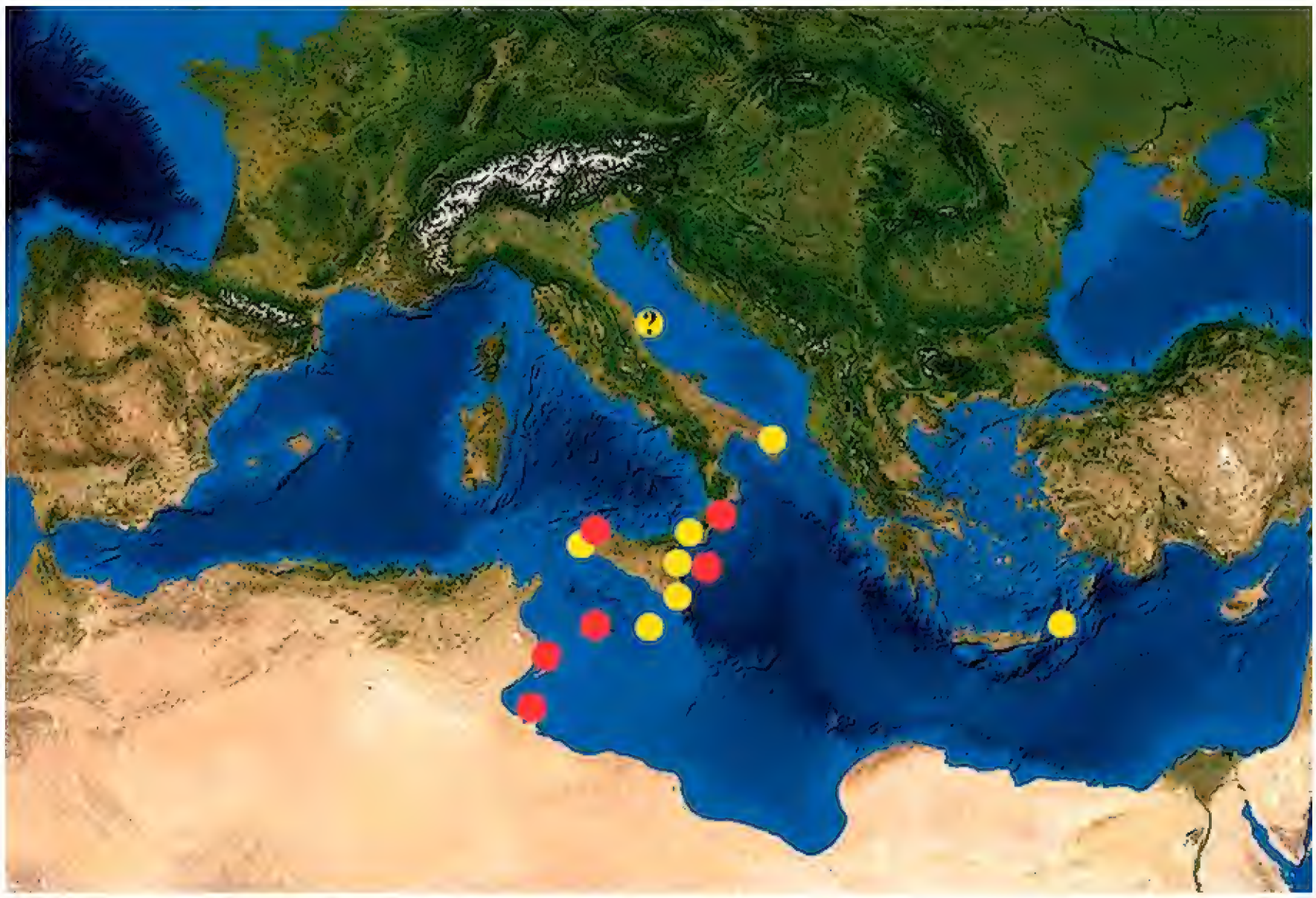


Figure 1. Map of the sampled area: red circles refer to *P. radiata* findings, yellow circles to *P. fucata* findings. The "?" refers to doubtful provenance.

Nowadays throughout the Southern Central and Eastern Mediterranean *P. radiata* can be considered a well-established species.

But the genetic diversity of populations of this species in the basin seems to exhibit low values, as reported by Barbieri et al. (2015).

In this last years in Sicily consistent materials of another morphologically different population of *Pinctada* were found along the Eastern coast (Villari & Scuderi, 2017), from Messina to Siracusa, in different environmental conditions compared to the quantitatively less important finding of *P. radiata*.

As suggested by these latter Authors, the model of settlement and spreading of this populations in these localities follows the model of a newly entered alien species. In fact specimens appeared in massive quantities in these localities where no other specimen of *Pinctada* since XIX century was previously recorded, with the exception of only one single shell in the harbor of Catania (Di Geronimo, 1971).

The Northern and the most Southern part of the island was reached only in a second time, with less consistent populations, being the harbor of Catania identified as the location of first settlement, probably after human mediated transportation.

Currently the population of Eastern Sicily benefits a good state of health, since large number of specimens were found just in winter 2018.

In the present paper, the taxonomic conundrum previously reported was re-examined applying morphological taxonomy to the above mentioned materials of both the morphs of *Pinctada*.

The study of this materials allowed us to discriminate two different groups on account of shell morphology, anatomy of the external soft parts and environment.

The first could be grouped under the so far known taxon *P. radiata*, while, according to us, the second represents a different species which here we call *P. fucata*, after comparisons with Indo-Pacific material. So, the real question is more general and trespass the Mediterranean limits: are these two taxa only synonyms of a single very variable species or they could indeed be considered a different species? A resume of the literature data on biomolecular studies conducted to solve doubts on the identity of species of this complex are here furnished together with our new personal observations on the morphology of both the shell and the soft body parts.

Comments and personal interpretations of the taxonomical status of both these morphs are here reported as an attempt to give our contribution to their complicated taxonomy.

MATERIAL AND METHODS

A total number of 1284 living specimens and shells of both the morphs were examined from many different Mediterranean localities, from central N-African coasts to the more Eastern places of the basin (Fig. 1), and grouped according to the collection site in the Mediterranean, studying the variation inside populations.

More accurate data are furnished in Table 2 for each morph debated in the following lines.

The most abundant population is that from the sandy coast of Catania (E-Sicily), where numerous alien species of animals and algae are constantly found during these last years.

Living specimens were found on a removable containment boom, which was installed on June 15 2018 and removed on September 18 of the same year. Some Indo-Pacific specimens of *P. fucata*, from Maldives Islands, were studied for morphological comparisons.

Specimens were measured and the shell morphology was studied: nomenclature of characters follows Wada & Temkin (2008).

We utilized as valid characters: the general outline, the sculpture and the prevalent colour of valves, the hinge structure and the shape of ligament and ligament area.

Living specimens were housed in aquarium and dissected: specimens of the two morphs were comparable in size (Figs. 39, 41).

Anatomical studies concerned the presence and shape of the anal funnel and colour of soft parts.

Table 1 lists the main characters utilized to distinguish *P. radiata* and *P. fucata* used in this study.

ACRONYMS AND ABBREVIATIONS. AGC: Alfio Germanà Collection, Catania, Italy; AVC: Alberto Villari Collection, Messina, Italy; DSC: Danilo Scuderi Collection, Catania, Italy; PBC: Paolo Balistreri Collection, Favignana, Trapani, Italy; PMC: Pasquale Micali Collection, Fano, Italy; WRC: Walter Renda Collection, Amantea,

Cosenza, Italy; sh.: empty shell/s; sp.: living collected specimen/s.

RESULTS

Systematics

Classis BIVALVIA Linnaeus, 1758
Subclassis PTERIOMORPHIA Beurlen, 1944
Ordo OSTREIDA Férussac, 1822
Superfamilia PTERIOIDEA Gray, 1847 (1820)
Familia PTERIIDAE Gray, 1847 (1820)

Pinctada Röding, 1798

TYPE SPECIES: *Pinctada margaritifera* (Linnaeus, 1758). Here follows the description of the main diagnostic characters observed for each of the two taxa treated in the present paper.

Pinctada radiata (Leach, 1814)
(Figs. 2–7, 17–19, 23, 35, 36, 39, 40)

EXAMINED MATERIAL. A total of 84 sh and 48 sp from different localities from Italy and Tunis (see Table 2 for details).

DESCRIPTION. Shell almost rounded in outline, variable in thickness and inequilateral (Fig. 27), high up to 75 mm, constituted by almost flat valves, the left more convex than the right one.

Outer face reddish with darker radial rows, sculptured by numerous concentric growth lamellae which bear, on the lower 1/2 or 1/3 of the valve, a copious number of pointed and narrow processes arranged in two orders: one larger alternated to one or two smaller (Fig. 27).

In the posterior margin of both the valves the rows are closer. The smooth inner surface is divided in a marginal less wide non-nacreous layer, which recalls the outer surface in colour, separated by a darker more or less wide band from the nacre one, which bears a wide adductor muscle scar and a series of small pallial muscle scars.

The right valve shows a not very marked rounded byssal ridge. The hinge bears a well-defined and duplicated posterior tooth and a not very well defined but thick anterior tooth with two not deep sockets.

The left valve shows a very marked rounded byssal ridge and bears a straight and clearly duplicated anterior tooth; posterior tooth not marked.

Straight hinge line, which become almost deeply curved near the umbo. Seen from inner side, the umbo is prominent in the left valve, almost invisible in the right one.

Anatomy of soft parts: digestive gland, kidney, gonad and ctenidium deep orange (Fig. 35); foot whitish with numerous black spots; ctenidium and margin of mantle orange with white stains and black radial bands corresponding to the marginal tips; anal funnel (Fig. 36) speare-shaped and graysh. Byssus green with almost thick filaments (Fig. 40).

DISTRIBUTION AND BIOLOGY. Quite common in Eastern basin, from Israel, Crete, Grece to Tunisia, Eastern and Western Sicily and S-Apulia. Tyrrhenian records should be confirmed with live-collected specimens.

Calcareous to lava hard substrata, with dense algal turf, at low depths (-0.5/8 m).

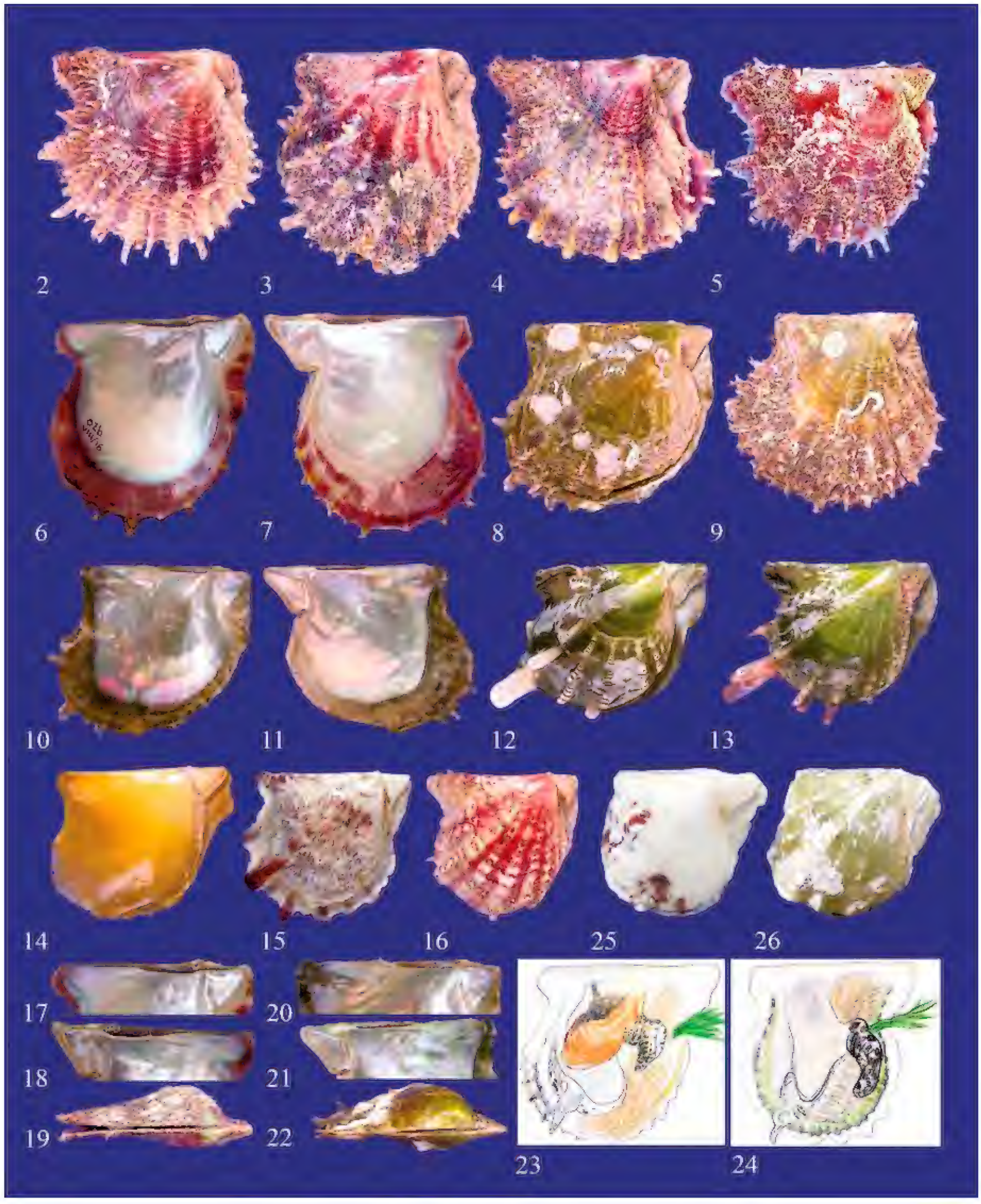
Pinctada fucata (Gould, 1850)
(Figs. 8–26, 20–22, 24, 37, 38, 41–50)

EXAMINED MATERIAL. A total of 761 sh and 392 sp from different localities from Italy, Greece and Malta (see Table 2 for details). Maldives: Ari Atoll, Kuda Rah, 96 Km S of Male, beached, 5 sh (DSC).

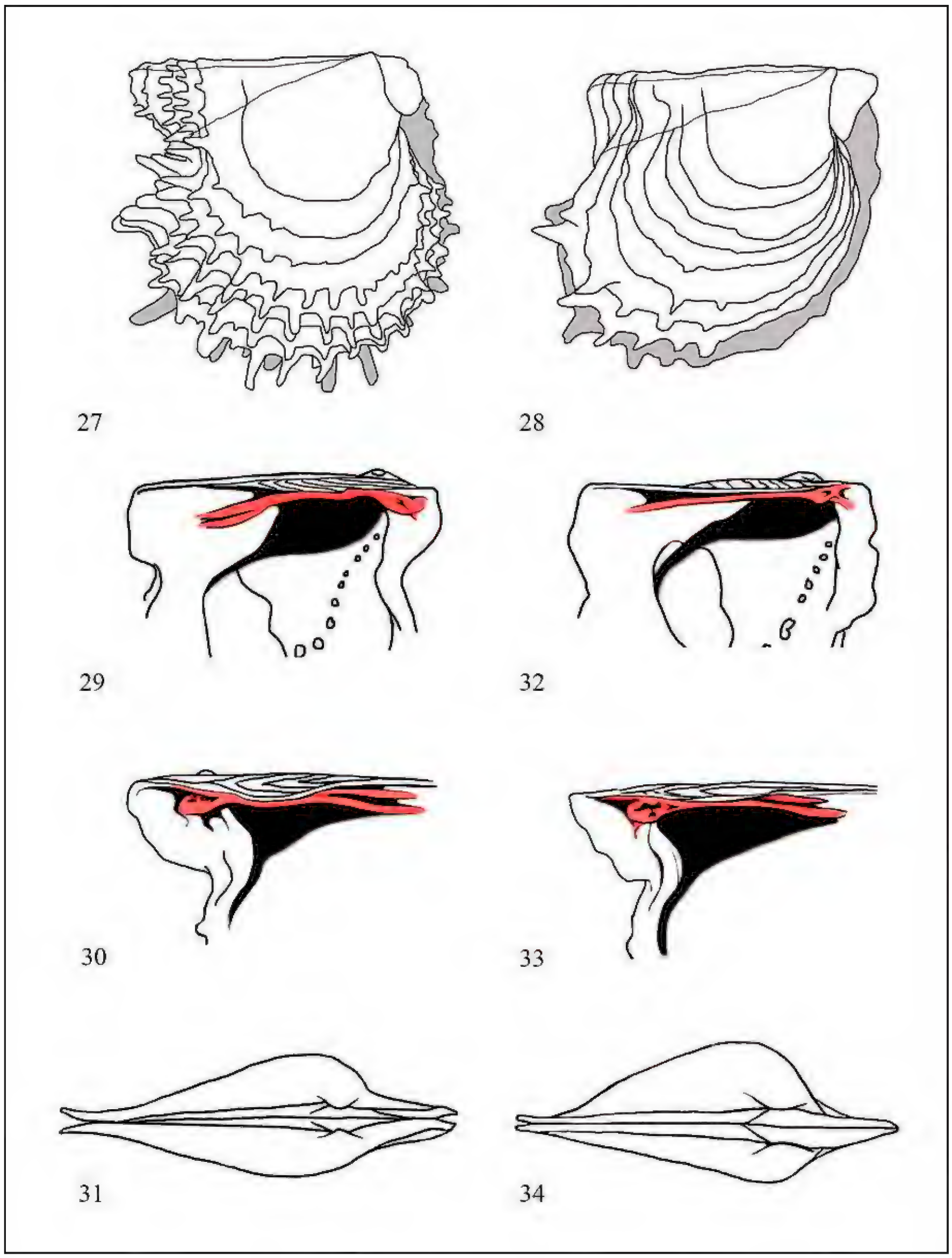
DESCRIPTION. Shell almost transverse-oval in outline, fragile and highly inequilateral (Fig. 26), high up to 45 mm, with a flat right valve and a left quite convex one. Colour very variable, from almost white specimens with darker stains to yellow, red, but the most common is green, within its wide range of variations, from paler to very dark, with whitish radial rows.

It is sculptured by concentric almost smooth growth lamellae, which bear, on the lower 1/3 or 1/4 of the valve, a low number of blunt and large processes arranged in two orders of 10–12 alternated rows of bigger and smaller processes (Fig. 26).

In the posterior margin of both the valves the rows bear bigger processes: those of the 2nd or 3rd row could form very long and large scales which stand up over all the others (Figs. 12, 13).



Figures 2–7, 17–19, 23. *Pinctada radiata*. Figs. 2–7: shells in external and internal view, all from Catania. Fig. 2: h: 59 mm. Fig. 3: h: 49 mm. Fig. 4: h: 42 mm. Fig. 5: h: 32 mm. Figs. 6, 7: h: 53 mm. Fig. 23, same data of the specimen in figure 4, drawing of the internal soft parts. Figs. 17–19, same data of the specimen in figures. 6, 7, detail of the hinge of the left (Fig. 17) and right (Fig. 18) valves and view from umbo (Fig. 19). Figures 8-16, 20–22, 24. *Pinctada fucata*. Figs. 8–16: shells in external and internal view, all from Catania. Figs. 8, 10–11: h: 46 mm. Fig. 9: h: 75 mm, corresponding to a possible hybrid specimen. Fig. 12: h: 20 mm. Fig. 13: h: 18 mm. Fig. 14: h: 31 mm. Fig. 15: h: 23 mm. Fig. 16: h: 26 mm. Figs. 20–22, same data of the specimen in figures 10, 11, detail of the hinge of the left (Fig. 20) and right (Fig. 21) valves and view from umbo (Fig. 22). Fig. 24: same data of the specimen in figure 41, drawing of the internal soft parts. Figs. 25, 26: Maldives Is., Ari atoll, h: 19 mm and h: 10 mm, respectively.



Figures 27, 28. Sketches of the shell outline respectively of *Pinctada radiata* and *P. fucata* (in gray margins of the lower valve). Figures 29–34. Sketches of the hinge in the left and right valve (in red details of the teeth) and shell outline seen from umbo of *P. radiata* (Figs. 29–31) and *P. fucata* (Figs. 32–34).

Figures 35, 36, 39, 40: *Pinctada radiata*. Fig. 35, photograph of the internal soft parts of the specimen in figure 39, h: 42 mm. Fig. 36, detail of the anal funnel. Fig. 40, byssus, same data of figure 35. Figures 37, 38, 41–50: *Pinctada fucata*. Fig. 37, photograph of the internal soft parts of the specimen in fig. 41, h: 40 mm. Fig. 38, detail of the anal funnel. Fig. 42, byssus, same data of fig. 37. Figs. 43–50, living specimens attached to various substrata from Catania "Playa". Fig. 43, group of specimens attached each other. Fig. 44, specimens on valves of *Acanthocardia tuberculata.* Fig. 45: specimens on a valve of *Fulvia fragilis* and the shell of *Neverita josephinae*. Fig. 46: specimens on the shell of a small *Pinna nobilis.* Fig. 47: a specimen on the tube of *Sabella spallanzanii.* Fig. 48: specimens on a rhizome of *Cymodocea nodosa*. Fig. 49: a specimen on *Caulerpa taxifolia* var. *distichophylla.* Fig. 50, a specimen on an ascidian of the genus *Microcosmus*.

The right valve shows a not very marked rounded byssal ridge.

The hinge bears a well-defined and duplicated posterior tooth and a not very well defined but thick anterior tooth with two often marked sockets.

The left valve shows a very marked rounded byssal ridge and bears straight and not duplicated anterior tooth; posteriorly an almost deep socket with two not market teeth is present.

Straight hinge line, which do not or only slightly become curved near the umbo. Seen from inner side, the umbo is prominent in the left valve, almost invisible in the right one.

Anatomy of soft parts: digestive gland, kidney and gonad pale orange to green, ctenidium greenish with white stains (Fig. 37); foot whitish with numerous black spots; margin of mantle deep green to pale orange, with white stains and black radial bands corresponding to the marginal tips, anal funnel graysh and speare-shaped (Fig. 38). Byssus green with almost thick filaments (Fig. 42).

DISTRIBUTION AND BIOLOGY. Sporadically reported in Eastern basin, in these last few years it is very abundant in Eastern Sicily and quite common in other Southern regions in Italy. The Adriatic record is reported *fide* the statements of the mariculture operators in Faro lake, Messina and should be confirmed. Sandy bottoms near estuarine areas, attached with byssus each other (Fig. 43) or on other shell, like mussels, Cardiidae (Fig. 44), Pinnidae (Fig. 46) and dead gastropods (Fig. 45), on ascidians (Fig. 50), on Annelid tubes (Fig. 47), to the stolons of the sea-grass *Cymodocea nodosa* (Ucria) Ascherson, 1870 (Fig. 48) or the algae *Caulerpa taxifolia* var. *distichophylla* (Sonder) Verlaque, Huisman et Procaccini in Jongma et al., 2013 (Fig. 49), but on plastic objects too, in low (-1/8 m) depths.

DISCUSSION

After a critical evaluation of the morphological characters observed in very abundant amount of specimens of the two different forms of *Pinctada*, despite a high variability in both and the genetic studies of mainly Eastern Mediterranean specimens (Barbieri et al., 2015), we concluded that two different species are involved

The first, attributable to *P. radiata*, on account of a bigger shell in full grown specimens, rounded in outline (Fig. 25), almost entirely red-brownish with darker radial strips, sculpture of dense and pointed process, organized in numerous rows, soft parts mainly orange (see Table 1).

The second is here named *P. fucata*, on account of comparisons with Indo-Pacific materials (Figs. 23, 24), and is characterized by a smaller shell in full grown specimens, oval in outline (Fig. 26), almost greenish in colour (75–80% of the specimens observed) with paler radial strips, sculpture essential, tending to vanish (valves often almost smooth), process sparse, blunt and wider, organized in low number of rows, soft parts mainly greenish. The former species, seen from umbo, is quite flattened (Fig. 31), while the latter appears markedly convex.

Besides the morphological differences above mentioned, *P. fucata* differs in habitat preferences. While *P. radiata* is present, nowadays with dense populations in Southern regions of East and Central Mediterranean, on hard substrata with algal covering (*Cystoseira* spp.), *P. fucata* was instead found near river's estuary on other shells, but the most abundant material is represented by vegetal residuals of the river cane *Phragmites australis* (Cav.) Trin. ex Steud., accumulated by winter storms. Less commonly it was found on the ascidians of the genus *Microcosmus* Heller, 1877 (Ascidiacea Pyuridae).

Considering the complicated taxonomy and the high polymorphism of the species of *Pinctada*, all these morphological differences are, according to us, enough to consider the two different morphs here studied as different species. We therefore refer to the conclusions chapter the full reasons of our personal interpretation of the taxonomical problem concerning these two taxa.

Moreover, in E-Sicily *P. fucata* was sporadically collected or never recorded before, in the same locality where thousands of specimens have been found in very recent times.

This population shows preferences for more brackish waters and sandy bottoms, where is more likely arrived with human mediated transportation, as its population dynamics seem to suggest.

The current status of the above reported populations of *P. fucata* in E-Sicily seems to attest their progressive expansion, after a first period of acclimation in the more suitable geographical zones where they arrived.

Characters	*P. radiata*	*P. fucata*
Shell outline	rounded	ovate
Dimensions (height of the valve)	up to 75 mm	up to 45 mm
Shell colour	red-brownish with darker vertical strips	greenish with paler vertical strips
Anterior tooth of the left valve	duplicated	Not duplicated
Hinge line	Curved near umbo	Straight
Ligament area	Narrow	Wide
Sculpture	numerous rows of dense and pointed processes	low number of rows of sparse, blunt and wider processes
Soft parts colour	Mainly orange	Mainly greenish
Environment	Hard substrates	Sandy bottoms near estuarine areas

Table 1. Comparison of morphological characters in *P. radiata* and *P. fucata*.

Specimens collected on a removable containment boom allowed us to evaluate the growth rate of the species: being the net completely clean at its first installation, after four months, among fouling, four specimens were found.

The medium grown rate of this species in the E-Sicily is 5.75 mm per month, which leaves us to believe that its expansion process in the Mediterranean Sea is very fast and facilitated by human activities.

CONCLUSIONS

According to differences in morphological characters of the shell and soft parts the two populations of Sicilian *Pinctada* are here considered different.

One, which we call *P. radiata*, has ever been recorded in scattered localities along the Sicilian rocky shores.

The second species, previously officially never recorded, appeared here only during the last 5-6 years, showing a population dynamic typical of an alien species.

But, if all the specimens constituting the large population reported as *Meleagrina* sp. by Monterosato (1878) from Alexandria belong to the same species figured in Appolloni et al. (2018), this species was the earliest lessepsian species spreading in the Mediterranean.

The disagreement of the present conclusions with previous molecular studies (Barbieri et al., 2015) according to us is attributable to the low number of specimens tested in these latter.

Probably previous records of *P. radiata* could be related to *P. fucata* too, like that of Crocetta et al. (2009), who found "*hundreds specimens*" along the Calabrian shores, among which some at list were *P. fucata* (specimens figured in Crocetta et al., 2009 at figures 2O, Q).

This latter species is present in Aegean Sea, as could be argued by pictures furnished by Manousis

Species	Locality and notes	coll.	sp	sh	Tot
P. fucata	Italy, Catania, Playa, beached, 2014-2018	DSC	382	736	
	Italy, Catania, S. Giovanni Li Cuti, rocky bottom, -2/8m, summer 2015-18	DSC	4	4	
	Italy, Catania, Cannizzaro, rocky bottom, -2/8m, summer 2016	DSC		2	
	Italy, Messina, Faro lake, among mussels from Rimini (Adriatic), as stated by farm workers	AVC	3		
	Italy, Messina, Contrada Paradiso, *Posidonia* beds -7m	AVC	3	12	
	Italy, Siracusa, Agnone, beached, III/2018	DSC	2	4	
	Italy, Siracusa, Porto grande, mouth of the Anapo river, 2017	DSC	2	3	
	Italy, Siracusa, Calabernardo, 1995-2015	DSC		3	
	Italy, Siracusa, Terrauzza, 1998	DSC		1	
	Italy, Trapani, Favignana, Cala Rotonda, -2m, 2017	PBC	1	2	
	Italy, Trapani, Ronciglio, beached, 2016	PBC		5	
	Greece, Is. Karpathos, -35m	PMC-AGC		2	
	Cyprus N-E, -12m	AGC	2		
	Malta: Ghar id-Dud, beached	DSC		1	
			397	775	1172
P. radiata	Italy, Catania, S. Giovanni Li Cuti, rocky bottom, -2/8m, summer 2013-2018	DSC	15	36	
	Italy, Catania, Cannizzaro, -2/8m, summer 2017-2018	AGC	11		
	Italy, Agrigento, Lampedusa, rocky bottom, -2/6m, 1989-2002	DSC		14	
	Italy, Agrigento, Linosa, rocky bottom, -2/6m, 1995	DSC	1	1	
	Italy, Lecce, Torre Guaceto, rocky bottom, 2010	DSC		2	
	Italy, Reggio Calabria, Saline Joniche Harbour, on aquacolture nets, november 2008	WRC		8	
	Tunisia, Djerba, El-Kantra, rocky bottom, -2/6m, april 2007	AGC	9	15	
	Tunisia, Kerkennah Is., rocky bottom, -2/6m, summer 2014	AGC	12	8	
			36	76	112
				Total	1284

Table 2. Materials of *P. radiata* and *P. fucata* utilized in the present study.

& Galinou-Mitsoudi (2013: fig. 4B), and is in rapid spreading in the Mediterranean, with large populations in certain localities, as could be argued by the growth rate of the species here attested.

Different species of *Pinctada* in the world seem to live in different geographical districts, but genetic differences between species seem not very high and molecular studies appeared controversial.

Moreover in geographical zones where the distribution areas overlap, morphological intermediates appear, adding confusions both to morphological and genetic studies.

Among the large amount of materials studied, we have found few intermediates between *P. radiata* and *P. fucata* as well (Fig. 9). So, how can we interpret these intermediates?

On the one hand, intermediates could represent morphs resulting by mating of different morphs of one only quite polymorphic species, which often live in different geographical areas.

But populations constituted by these morphologically different specimens tend to remain quite constantly uniform with respect to those of other geographic regions.

So, another interpretation is that they represent different species, which could hybridize themselves when populations overlap their distributional areas.

So, these intermediates represent hybrid specimens between two species.

Among bivalve molluscs, Mytylidae comprise examples of the same problem.

Genetic studies attested the existence of three different european species of *Mytylus*: *M. galloprovincialis* in the Mediterranean Sea and adjacent areas, *M. edulis* in North-Western coasts of Europe, and *M. trossulus* along Scandinavian coasts (Koehn, 1991).

Intermediates between these species occur along overlapping ridges of geographical distribution areas (Gardner et al., 1993; Gosling et al., 2008).

Mytylidae is a family of bivalves phylogenetically similar to Pteriidae.

According to us, the case of the Mediterranean *Pinctada* hybrids is similar to that of the *Mytylus* species. Moreover, populations of different species, from different geographical areas hybridize themselves.

ACKNOWLEDGEMENTS

We are grateful to Bruno Amati (Roma, Italy), for interesting comments and for literature data furnished. Pasquale Micali (Fano, Italy), Walter Renda (Amantea, Italy) and Alberto Villari (Messina, Italy) are thanked for their helpful comments and loan of materials. The supervisors, Emanuele and Giuliano D'Arrigo, and the lifeguard team of the beach resort "Cocoa Beach" of Catania, Italy (Salvo Costanzo, Mirko Campo, Carmelo Pulvirenti and Carlo Esterini) are thanked for stimulating one of the Author (DS) to pick among the removable containment boom.

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Biodiversity Journal, 2019, 10 (4): 427–430 https://doi.org/10.31396/Biodiv.Jour.2019.10.4.427.430
MONOGRAPH

The Biobank of the "Istituto Zooprofilattico Sperimentale" of Sicily (Italy): an important resource in medical research for safe and quality storage of biological specimens

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ABSTRACT The "Biobanca del Mediterraneo" (BBM) has been recently developed at the Istituto Zooprofilattico Sperimentale (IZS) of Sicily in Palermo, with the aim to collect and store under standard conditions and in a centralized system several types of certified animal and zoonotic biological resources (bacterial and viral strains, parasites, nucleic acids, positive/negative sera, cell cultures, tissues) in accordance to Quality System procedures as regulated by UNI CEI EN ISO/IEC 17025:2018. Presently, biological material from the OIE Reference Laboratories and National Reference Centres of the IZS of Sicily are being stored. Before storage, selection and cataloging of the most representative biological material collected from the Institutes' laboratories is performed. The "Biobanca del Mediterraneo", together with four other IZSs (IZS Emilia Romagna e Lombardia, IZS Piemonte, Liguria e Valle d'Aosta, IZS Venezie e IZS Abruzzo e Molise), is part of the Network of "Biobanche Veterinarie", recognised as "OIE Collaborative Center for Veterinary Biological Biobank" by the "Office International des Epizooties" (OIE). The objectives of the Biobank are the promotion and implementation of collaborations with the scientific community in order to harmonize and standardize biobanking practices and the development of scientific and technological research to provide services to both the scientific and business world. In fact, collected samples can be used for diagnosis, research, vaccine and drug production, epidemiological studies and other applications.

KEY WORDS Biobanca del Mediterraneo; veterinary biobank; biological resources.

Received 28.11.2019; accepted 19.12.2019; published online 20.12.2019
Proceedings of the 4th International Congress on Biodiversity "Man, Natural Habitats and Euro-Mediterranean Biodiversity", November 17th-19th, 2017 - Malta

INTRODUCTION

The "Biobanca del Mediterraneo" has been recently developed at the Istituto Zooprofilattico Sperimentale (IZS) of Sicily in Palermo, with the aim to collect and store under standard conditions and in a centralized system several types of certified animal and zoonotic biological resources in accordance to Quality System. Since 2014, the "Biobanca del Mediterraneo" together with other 4 II.ZZ.SS. (IZS Emilia Romagna e Lombardia, IZS Piemonte, Liguria e Valle d'Aosta, IZS Venezie, IZS Abruzzo e Molise), is part of the Network of "Biobanche Veterinarie" (Bontempi et al., 2019). Up to now, biological material from the OIE Reference Laboratories (Babesiosis; Theileriosis; Leishmaniosis, Contagious

agalactia) and the National Reference Centres (C.R.A.Ba.R.T.: Anaplasma, Babesia, Rickettsia, Theileria; C.Re.N.A.: Anisakiasis; Ce.Tox.: Toxoplasmosis; C.Re.Na.L.: Leishmaniosis; C.Re.Ta.M.: Wellbeing, Monitoring and Diagnosis of Diseases of Sea Turtles) of the IZS of Sicily (Fig. 1) have been stored. The storage activity is implementing thanks to the selection and cataloging of the most representative biological materials collected from the laboratories of the Institute and destined, for their intrinsic characteristics, to enrich the Biobank.

The main goals of the BBM are:

- adopt and maintain in the processes an effective Quality Management System, in products, in services, in compliance with the prescriptive requirements of the regulations and with any and other requirements that BBM decides to endorse;
- ensure the resource availability, the necessary information and knowledge for the operation and control of processes, through regular training activities aimed at informing employees of the relevance and importance of their activities and the ways to achieve the objective;
- understand and strengthen the relationship with customers and other stakeholders, improving their level of satisfaction through products and services in line with expectations;
- standardization of storage times of different "biological resources" and their traceability;
- improving the quality of the stored "Biological resources" according to the OIE guidelines;
- select and qualify the products suppliers and services that have an impact on the final quality processes and products, in the achievement of the company's objectives;
- identify the needs of new technological innovation to develop new services and processes according to the expectations of the scientific community;
- draw up an online easy and accessible catalogue for all interested parties;
- endorse the Memoranda of Understanding to comply with the original intellectual property and availability of use;
- carry out all assessments on the basis of objective evidence and in compliance with the rules and regulations in use.

The Managment of the Biobanca del Mediterraneo is committed to implementing and maintaining a quality policy for output quality services of: conservation of biological material and related data, best defined as "biological resources", transfer of "biological resources", production of certified reference materials. In order to achieve and maintain its objectives over time, BBM adopts a quality management system complying with the UNI EN ISO 9001. The criteria of "biological resources" stored in BBM respect the system and test procedures in accordance with ISO/IEC 17025 (General requirements for the competence of testing and calibration laboratories).

MATERIAL AND METHODS

Numerous matrices (bacteria, nucleic acids, parasites, positive/negative sera, stem cells, anatomical

Figure 1. The OIE Reference Laboratories and the National Reference Centres of the IZS of Sicily.

specimens) were collected and stored in order to be shared with scientific community. All the samples were checked according to UNI CEI EN ISO/IEC 17025:2018 to ensure their identity, quality and purity (Di Bella et al., 2018; Di Marco et al., 2010). Before storage, selection and cataloging of the most representative biological material collected from the laboratories of IZS were performed. All resources were accompanied by the following documents: Safety Data Sheet, Recovery Sheet, Certificate of Analysis containing product identification, qualitative and quantitative traits, reference to the used analytical method, and signature of the director, Dangerous Goods Declaration. Users interested in biological resources stored in the biobank can search the material of interest in the BBM catalogue selecting the material's code to download the technical sheet. The material may be requested and obtained after completion of the Material Transfer Agreement (MTA). The web site dedicated to the Biobanca del Mediterraneo is in continuous development and updating (Fig. 2).

RESULTS

From 2014 to 2016, IZS of Sicily has stored about 50 specimens. An identification sheet accompanied each sample with all the data concerning quality controls and biosecurity. Currently all information on samples stored in biobank are available at the BBM site (www.bbmed.it). Visiting the website it is possible to know the number of available samples, their origin, laboratory of preparation/isolation and temperature of storage.

The BBM included some of the most modern technological systems for the continuous maintenance and monitoring of the temperature of conservation to -20, -80, -196°C, a video surveillance systems and access control. Electronic systems ensure the verification of the optimal environmental parameters for best environmental performance and personnel safety.

Biobank activity is in continuous enrichment because new biological resources will be included in its biorepository.

DISCUSSION AND CONCLUSIONS

Biobank tasks regard the collection and sharing of bio-specimens with well defined features and associated data (Botti et al., 2008; De Paoli, 2005; Henny, 2003; Lombardo et al., 2015; Quinlan et al., 2015; Regulations European Biobank Maastricht, 2003; Vaught et al., 2011), but also the storage of biological material for the study and the protection of biodiversity (Costa et al., 2018) (Fig. 3). Indeed BBM has specific storage areas for the collection of genetic material from flora and fauna and it also offers medium- and long-term storage services to third parties.

The objectives of the Biobank are the promotion and implementation of collaborations with the scientific community in order to harmonize and standardize bio-banking practices, according to international OIE guidelines and the development of scientific and technological research to provide services to both the scientific and business world. In fact, collected samples can be used for diagnosis,

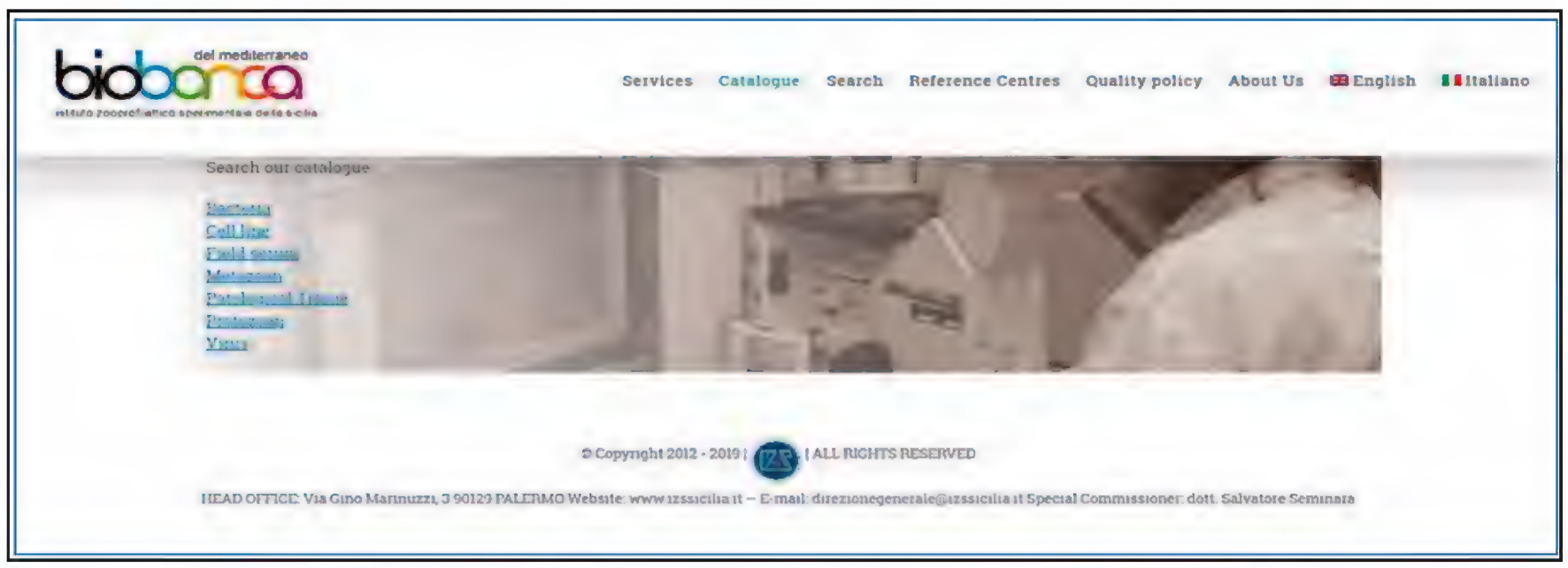

Figure 2. Page of the Biobanca del Mediterraneo website dedicated to the catalog.

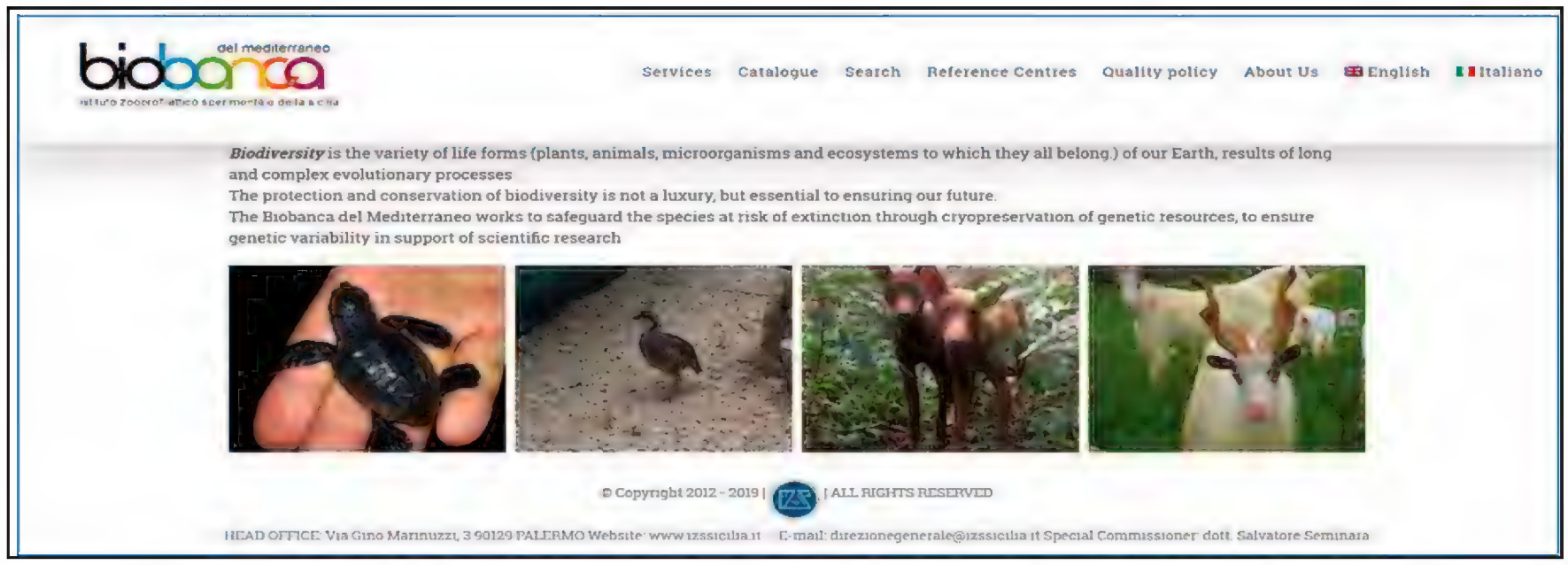

Figure 3. Page of the Biobanca del Mediterraneo website dedicated to the storage of biological material for the study and the protection of biodiversity.

research, vaccine and drug production, epidemiological studies and other applications. The "Biobanca del Mediterraneo" represent an unique structure throughout Southern Italy and thank to its potentiality to develop scientific research and technological innovation could become an international reference center (Guercio et al., 2008).

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Biodiversity Journal, 2019, 10 (4): 431–436 https:// doi.org/10.31396/Biodiv.Jour.2019.10.4.431.436
MONOGRAPH

Viral encephalopathy and retinopathy (VER) in Mediterranean wild and farmed fish species: the experience of the Istituto Zooprofilattico Sperimentale of Sicily (Italy)

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ABSTRACT *Betanodavirus* infection is widespread in a broad spectrum of fish species worldwide. In Italy, it is responsible for outbreaks of Viral Encephalo-Retinopathy (VER) that causes mortality and economic losses in sea fish farming. The infection is also widespread in wildlife, where there are generally no observed clinical manifestations. In this study we report the results obtained from the decennial activity of Istituto Zooprofilattico Sperimentale of Sicily on the research of *Betanodavirus* infection in wild fish of Mediterranean Sea and in farmed fish. Among the fish species analyzed, *Gobius niger* (Linnaeus, 1758), *Mullus barbatus* (Linnaeus, 1758), and *Trisopterus minutus capelanus* (Lacepède, 1800) were found positive and these could be a reservoir in which the virus can survive for long periods of time. The *Betanodavirus* isolation from pelagic species such as *Pagellus erythrinus* (Linnaeus, 1758), *Sardina pilchardus* (Walbaum, 1792), *Lepidopus caudatus* (Euphrasen, 1788), *Epinephelus marginatus* (Lowe, 1834), *Epinephelus aeneus* (Geoffroy Saint-Hilaire, 1817) resulted interesting because these species could play a more significant epidemiological role, being able to move even at distances.

KEY WORDS Viral encephalopathy and retinopathy (VER); *Betanodavirus*; sea fish; farmed fish.

Received 28.11.2019; accepted 19.12.2019; published online 20.12.2019
Proceedings of the 4th International Congress on Biodiversity "Man, Natural Habitats and Euro-Mediterranean Biodiversity", November 17th-19th, 2017 - Malta

INTRODUCTION

Betanodavirus is non-enveloped, spherical and approximately 25 nm in diameter, RNA virus belonging to the family of Nodaviridae (Schneemann et al., 2005). The genome consists of two molecules of positive-sense ssRNA: RNA1 (3.1 kb) encodes the replicase (110 kDa) and RNA2 (1.4 kb) encodes the coat protein (42 kDa). Since 1995 the ERV has also been reported in Italy where the most affected species is European bass, *Dicentrarchus labrax* (Linnaeus, 1758). It is responsible for outbreaks of Viral Encephalo-Retinopathy (VER) otherwise known as viral nervous necrosis (VNN), considered to be a serious disease of several marine fish species, characterised by significant losses associated to vacuolating lesions of the central nervous system and the retina. To date, the disease has been reported in more than 50 fish species, mainly marine with the greatest impact being in striped jack, European sea bass (*Dicentrarchus labrax*), groupers, and flatfishes (Munday et al., 2002; Sano et al., 2011).

VER causes mortality and economic losses in sea fish farming; the infection is also widespread

in wildlife (Gagnè et al., 2004; Guercio et al., 2004), where there are generally no observed clinical manifestations. A few outbreaks have also been documented in freshwater farms (Bovo et al., 2011; Chi et al., 2003) suggesting that salinity is not a limiting factor and it is possible that the virus may spread to economically important freshwater species. Interspecies transmission has been demonstrated and the presence of asymptomatic carriers in wildlife is strongly suspected. The risk of horizontal transmission between wildlife and farmed fish is particularly high in fattening farming of marine fish that is generally conducted in sea cages or in brackish ponds where there is high possibility of contact with natural environment (Ciulli et al., 2007). Furthermore, the presence of *Betanodaviruses* was detected in bivalve molluscs. This invertebrates when reared in the same area as farmed and wild finfish could act as a reservoir of the virus. Current European regulations allow relaying activities and the sale of live bivalve molluscs, which could pose a real risk of spreading *Betanodaviruses* across different geographic regions (Volpe et al., 2017). Knowledge regarding the extent of disease spread is fundamental for its direct prophylaxis and control.

In this study, we reported the results obtained from the decennial activity of Istituto Zooprofilattico Sperimentale (IZS) of Sicily on the research of *Betanodavirus* infection in wild fish of Mediterranean Sea and in farmed fish (Ciulli et al., 2005; Ciulli et al., 2006a; Ciulli et al., 2006b; Guercio et al., 2004; Nishizawa et al.,1994; Purpari et al., 2007; Toffan et al., 2014).

MATERIAL AND METHODS

Between 2004 and 2016, a total of 1614 tests for *Betanodavirus* (RT-PCR and viral isolation on cell cultures) were performed on brain samples of wild-caught and farm-raised fishes along the coasts of Sicily (Italy).

Viral isolation on cell cultures

Brain samples were homogenised with 1:5–1:10 volumes of Hanks' balanced salt solution (HBSS, Sigma-Aldrich) containing antibiotics (penicillin - 800 International Units [IU]/ml and streptomycin - 800 µg/ml) to avoid bacterial contaminations. The antibiotic treatment was performed for 4 hours at 15°C or overnight at 4°C.

The antibiotic treated tissue suspension at two different dilution: the primary dilution in culture medium L-15 (Sigma-Aldrich) and a 1:10 dilution thereof, were inoculated on SSN-1 cells (cell line derived from striped snakehead (Frerichs et al., 1996) and incubated at a temperature of + 25°C. The cells were monitored by microscopy daily for 10 days in order to highlight the presence of a cytopathic effect (ECP) attributable to the presence of the virus. If no CPE occurred after the primary incubation period, subcultivation was performed on fresh cultures, using a similar cell growing area to that of the primary culture.

One Step RT-PCR

Viral RNA was extracted from brain samples and cell culture supernatants using the High Pure Isolation Kit (Roche) according the manufacturer's instructions. Total RNA was subjected to reverse transcription followed by PCR amplification performed by AccessQuickTM RT-PCR System (Promega) following the manufacturer's recommendations. A pair of primers, designated as OIEF2/ OIE-R3 (Table 1), specific for a 427 bp fragment of the T4 region of the RNA2 gene coding for the 42 KDa capsid protein (Nishizawa et al.,1994) was used. The thermocycling conditions were: + 54°C for 30 min, + 92°C for 2 min and 35 cycles of 30 s denaturation at + 94°C, 30 s annealing at + 55°C and 30 s elongation at + 72°C; the reaction was terminated with a 7 min elongation at + 72°C.

The methods used were consistent with what is described in the O.I.E. Manual (2013) and certified according to UNI EN ISO/IEC 17025:2018 quality standards.

PRIMERS	SEQUENCE
OIE-F2	5'-CGT GTC AGT CAT GTG TCG CT-3'
OIE-R3	5'-CGA GTC AAC ACG GGT GAA GA-3'

Table 1. Primers used in One Step RT-PCR.

RESULTS

Over a decade of activity, a total of 736 virus isolations on cell cultures (Table 2) and 878 RT PCR for *Betanodavirus* (Table 3) have been performed on brain samples of wild fish and breeding species from all over Sicily. A total of 88 samples were positive in RT-PCR and 126 by the culture method. The discrepancy in the results is due to the fact that it has not always been possible to analyze the samples with both methods; in particular, none of the 2004 samples and not all the 2005 samples were examined by the RT-PCR. SSN-1 cells inoculated with positive samples showed the presence of a cytopathic effect (ECP) attributable to the presence of the virus (Figs. 1, 2).

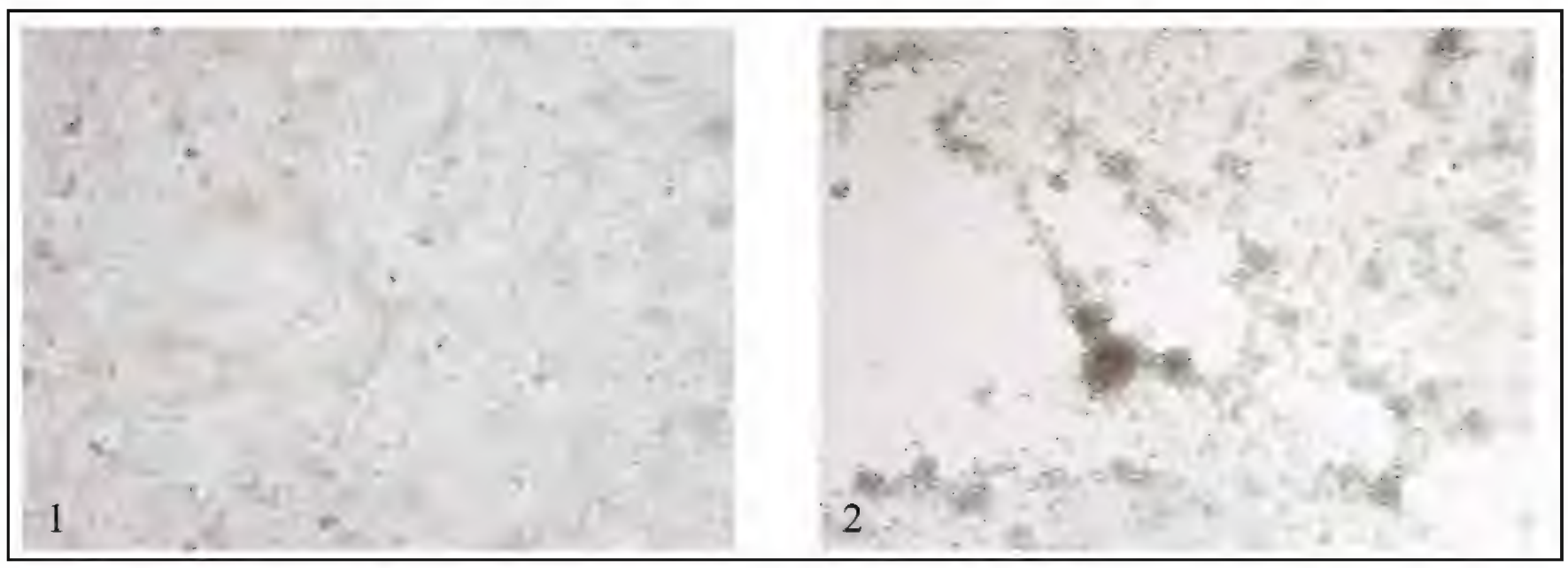
The "Fish, Crustaceans and Molluscs Disease Reference National Centre of the IZS Venezie" confirmed the positives. Among the species positive for *Betanodavirus* are commonly affected species such

YEAR	N°. TEST/YEAR	NEGATIVE	POSITIVE/FISH SPECIES	WILD/FARMED FISH
2004	5	3	*1 Pagellus erythrinus* *1 Trisopterus minutus capellanus*	wild
2005	369	307	50 *Gobius niger* 5 *Sardina pilchardus* 6 *Mullus barbatus* 1 *Trachiurus trachiurus*	wild
2006	97	97	0	
2007	99	99	0	
2008	97	97	0	
2009	11	3	8 *Epinephelus marginatus*	wild
2010	-	-	-	
2011	-	-	-	
2012	-	-	-	
2013	1	0	1 *Epinephelus aeneus*	wild
2014	57	4	2 *Epinephelus marginatus* 51 *Dicentrarchus labrax*	wild farmed
2015	-	-	-	-
2016	-	-	-	-
TOT	736	610	126	-

Table 2. Results of the VER isolation on SSN-1 cells from fish brains from 2004 to 2016.

YEAR	N°. TEST/YEAR	POSITIVE	NEGATIVE
2005	165	23	142
2006	58	0	58
2007	151	0	151
2008	225	2	223
2009	52	8	44
2010	15	0	15
2011	5	0	5
2012	3	0	3
2013	102	0	102
2014	58	53	5
2015	41	2	39
2016	3	0	3
TOT	**878**	**88**	**790**

Table 3. Results obtained in RT-PCR for VER performed on fish brains from 2005 to 2016.



Figures 1, 2. SSN-1 monolayer (Fig. 1) andECP induced by VER on SSN-1 cells (Fig. 2).

as *Dicentrarchus labrax*, wild benthic species such as *Gobius niger* (Linnaeus, 1758), *Mullus barbatus* (Linnaeus, 1758) and *Trisopterus minutus capelanus* (Lacepède, 1800) and wild pelagic species very common in the Mediterranean Sea such as *Pagellus erythrinus* (Linnaeus, 1758), *Sardina pilchardus* (Walbaum, 1792), *Lepidopus caudatus* (Euphrasen, 1788), *Epinephelus marginatus* (Lowe, 1834), *Epinephelus aeneus* (Geoffroy Saint-Hilaire, 1817). A total of 55 strains of *Betanodavirus* have been also cryo-preserved in liquid nitrogen.

DISCUSSION AND CONCLUSIONS

Since 2004, IZS of Sicily in collaboration with IZS delle Venezie is concerned with the research of *Betanodavirus*, highlighting its widespread diffusion in breeding species and in many wild species

of the Mediterranean Sea, in which, in most cases, the presence of virus had never been reported. Among the species that were positive, *Gobius niger*, *Mullus barbatus* and *Trisopterus minutus capellanus* can be reservoir in which the virus can survive for long periods of time. The isolation of *Betanodavirus* from pelagic species such as *Pagellus erythrinus*, *Sardina pilchardus*, *Trachiurus trachiurus*, *Epinephelus marginatus*, *Epinephelus aeneus* has aroused particular interest.

In fact, these species could play a more significant epidemiological role, being able to travel a large distance and these could easily be in touch with farmed fish, bringing infection from a farm to others. Such a wide spread of the pathogen, even in a natural environment, makes it necessary to investigate the existing epidemiological correlations between infection in farmed and wild species for the close contact between the two environments.

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Biodiversity Journal, 2019, 10 (4): 437–444 https://doi.org/10.31396/Biodiv.Jour.2019.10.4.437.444
MONOGRAPH

Detection of human enteric viruses in water and shellfish samples collected in Sicily (Italy)

Giuseppa Purpari, Santina Di Bella, Francesca Gucciardi, Francesco Mira, Santino Barreca, Laura Di Paola, Giusi Macaluso, Patrizia Di Marco & Annalisa Guercio

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ABSTRACT Enteric viruses are responsible for foodborne and waterborne infections caused by contaminated food products and water, affecting a large number of people. Among the foods an important role is played by shellfish, on the ground that they can be eaten raw or undercooked. Data on food and water viral contamination in South of Italy are scarce and fragmentary. As illnesses result from the failure to control an hazard, the aim of this study was to detect the main pathogenic human enteric viruses in the environment, for evaluating the presence of viral contamination in shellfish and water samples collected in South of Italy (Sicily). The survey was conducted over a period of five years (2012–2016) on 16 water samples (sea waters, pipe waters and torrent water), and 72 fresh and frozen shellfishes from harvesting areas, restaurants, and markets during regional official control or checked at Veterinary Border Inspection Posts. Hepatitis A virus (HAV), GI and GII genogroup Norwalk virus (NoVs), Adenovirus and Rotavirus were detected by nucleic acid amplification (end-point and Real Time PCR/RTPCR) and sequence analysis. The most frequently detected viruses in shellfish were GI NoV (16.7%) and HAV (18.0%). Of the 16 water samples 12.5% were positive for GII NoV and 6.2% for RoV. Molecular surveillance of water and shellfish clearly demonstrated that human pathogenic viruses are widely found in aquatic environments and confirmed the role of bivalve molluscs as main reservoirs.

KEY WORDS Enteric virus; PCR; Genotyping; Shellfish; Water.

Received 28.11.2019; accepted 19.12.2019; published online 20.12.2019

Proceedings of the 4th International Congress on Biodiversity "Man, Natural Habitats and Euro-Mediterranean Biodiversity", November 17th-19th, 2017 - Malta

INTRODUCTION

Numerous viruses of human or animal origin are found in the environment and infect people by water and food: bivalve molluscs along with vegetables and prepared foods are classified by the World Health Organization as priority hazards (WHO, 2018). Most of these viruses belong to the families Adenoviridae, Caliciviridae, Hepeviridae, Picornaviridae and Reoviridae (Dubois et al., 1997; Muscillo et al., 2001; Lodder & de Roda Husman, 2005). These pathogens are routinely introduced into the environment through the discharge of treated and untreated wastes that can be transported through groundwater, estuarine water, seawater, and rivers (Okoh et al., 2010; La Rosa & Muscillo, 2012; La Rosa et al., 2012). A succession of health alarms, "foodborne diseases" and "waterborne diseases", increasing the attention on food and water safety was decribed in the last decades (Le Guyader

et al., 2000; Pintó et al., 2009; EFSA, 2011, 2016; Sánchez & Bosch, 2016). Shellfish and water could play an important role in the determination of environmental contamination. Consumption of fish products, with particular reference to Edible Lamellibranch Molluscs (ELM), is a risk for human health because of their capacity to filter, accumulate and concentrate pathogens present in the contaminated water. Hepatitis A (HAV), Norovirus (NoV) and Rotavirus (RoV) and the emerging Hepatitis E (HEV) have been found in shellfish, especially mussels and oysters (Bellou et al. 2013). Viral waterborne disease outbreaks associated with contaminated drinking or recreational waters are reported worldwide, suggesting the contamination of aquatic environments (Kamel et al., 2011; Prado et al., 2012; La Rosa et al., 2015). Contaminated water could irrigate vegetables and retain enteric viruses on their surface (Petrinca et al., 2009; La Rosa et al., 2010a; Severi et al., 2015; Scavia et al., 2017; Iaconelli et al., 2016). HAV, NoV, Adenovirus (AdV), multiple strains of (EV) (Echoviruses and Coxsackievirus) and HEV are enteric viruses associated with human wastewater (Lodder et al., 2005).

EV is able to contaminate and survive in brackish and probably saltwater and in shellfish (Patel et al., 2009).

Hepatitis A virus is highly resistant in the environment, and typically transmitted through raw shellfish or water (Pintó & Bosch, 2013). The consumption of raw shellfish caused outbreaks in 1996-1997 and 2004 in southern Italian regions (Apulia and Campania) (Malfait et al., 1996). Iaconelli et al. (2015) assessed the occurrence of HAV in shellfish samples, detecting in 23.2% samples, 12 genotype IB and one genotype IA. La Rosa et al. (2017) investigated the spread of HAV in Italy through the monitoring of raw urban sewages and detected several variants with a prevalent IB strain having a countrywide distribution.

Norovirus can be classified into seven genogroups, GI to GVII (Vinjé, 2015; Zheng et al., 2006) with more than 30 genotypes within genogroups GI, GII, and GIV infecting humans (Kroneman et al., 2013). Several routes of NoV transmission have been identified in many well-documented outbreaks. Foodborne transmission, including infected foodhandler, can play an important

BIOMOLECULAR ASSAYS	
HAV RT-PCR	GeneAmp® RNA PCR Core Kit - Applied Biosystem (Le Guyader *et al.*, 1994)
HAV Real Time RT-PCR	UltraSense ™ One-Step (Quantitative) qRT-PCR System RNA kit - Invitrogen (ISO/TS 15216-2:2013; Costafereda *et al.*, 2006)
NoV RT-PCR	GeneAmp® RNA PCR Core Kit - Applied Biosystem (Vinje e Koopman, 1996)
GI and GII NoVs Real Time RT-PCR	UltraSense ™ One-Step (Quantitative) qRT-PCR System RNA kit – Invitrogen (ISO/TS 15216-2:2013; da Silva *et al.*, 2007)
Rotavirus Real Time RT-PCR	TaqMan Universal PCR Master Mix - Applied Biosystem (Freeman *et al.*, 2008)
Adenovirus PCR	Taq PCR Core Kit - QIAGEN (Formiga-Cruz *et al.*, 2005)

Table 1. Biomolecular assay amplification kits and references.

Year	Matrix	N. Samples	HAV Positive/Total	GI NoV Positive/Total	GII NoV Positive/Total	Adenovirus Positive/Total	Rotavirus Positive/Total
2012	Shellfish	11	3/11	5/11	0/11	0/11	0/11
	Water	/	/	/	/	/	/
2013	Shellfish	27	10/27	5/27	3/27	1/27	0/27
	Water	6	0/6	0/6	0/6	0/6	0/6
2014	Shellfish	24	0/24	2/24	1/24	1/24	0/24
	Water	4	0/4	0/4	1/4	0/4	1/4
2015	Shellfish	2	0/2	0/2	0/2	0/2	0/2
	Water	/	/	/	/	/	/
2016	Shellfish	8	0/8	0/8	0/8	0/8	0/8
	Water	6	0/6	0/6	1/6	0/6	0/6
TOTAL	*Shellfish*	*72*	*13/72*	*12/72*	*4/72*	*2/72*	*0/72*
	Water	*16*	*0/16*	*0/16*	*1/16*	*0/16*	*1/16*

Table 2. Detection of enteric viruses from shellfish and water samples.

role. In a meta-analysis of NoV outbreaks in nursing homes, foodborne introduction was described for 7% and only 0.7% of outbreaks was reported to be foodborne, 28.5% as person-to-person, and 70.8% remained unknown or not mentioned (Petrignani et al., 2015). Several waterborne outbreaks have been described, with an indirect evidence of potential airborne transmission (such as in explosive vomiting occurred during the disease) (La Rosa et al., 2012; Giammanco et al., 2014; Giammanco et al., 2018).

Rotaviruses infections are found worldwide. It is estimated to cause more than 200,000 deaths annually; in particular children are infected during the first 6–9 months of life (Soriano-Gabarró et al., 2006).

Human adenovirus (HAdV) are the only human enteric viruses to contain DNA. They are often detected with other human enteroviruses and/or hepatitis A virus in different environments (Puig et al., 1994; Pina et al., 1998). HAdV have been widely detected in wastewaters, surface waters, recreational waters as well as in treated and disinfected drinking water (Mena & Gerba, 2009). For these reasons it has been proposed as indicator (for monitoring human faecal water contamination and efficacy of water purification) (La Rosa et al., 2010b).

Among the wide range of enteric viruses, four groups - HAV, NoV, RoV and AdV- were selected for this study due to their epidemiological significance as foodborne and waterborne pathogens (EFSA, 2011, 2016; La Rosa et al., 2012; Sánchez & Bosch, 2016). The objective was to assess the presence of HAV, GI and GII NoV, AdV and RoV in bivalve molluscs and environmental waters by end-point and real-time PCR/RT-PCR and sequence analysis.

MATERIAL AND METHODS

A total of 72 fresh and frozen shellfish and 16 environmental waters, were collected between 2012 and 2016. All the samples were screened for HAV,

GI and GII NoV, AdV and RoV molecular detections.

Sampling

Shellfish samples were collected from restaurants and fish markets, from three harvesting areas in the province of Syracuse and five centers in Messina and during official control monitoring programs in Sicily. They consisted in different species: *Mytilus galloprovincialis*, *Mytilus edulis*, *Tapes semidecussatus*, *Tapes decussatus*, *Ensis directus*, *Crassostrea gigas*, and *Venus verrucosa*.

Water samples included brackish water from the mussel farming centre in Syracuse); pipe water from Santo Stefano di Quisquina, Agrigento, where a Norovirus outbreak had occurred in 2011 (Giammanco et al., 2014) and from the province of Catania where in May 2016 a NoV outbreak had occurred (Giammanco et al., 2018); water from a desalter and seawaters (water from Lampedusa island, Agrigento, where a HAV outbreak had occurred in 2014); torrent water from the province of Caltanissetta.

Preparation of molluscs

Each batch of mollusk sample consisted in 25 g of hepatopancreas homogenized with 0.05 M glycine buffer (pH 9.2) and processed with a double 1.5 M NaCl PEG8000 (final concentration of 12.5 %) precipitation (Croci et al., 1999). A parallel extraction from 2 g of hepatopancreas was performed using treatment with a proteinase K solution, following the International Organization for Standardization (ISO) technical specification (ISO 15216-2:2013).

The samples were spiked with 10 µl of titrated Mengovirus process control strain MC0 (1.6x104 TCID50/ml), to monitor extraction efficiency following the ISO 15216 guidelines.

Preparation of water samples

Water samples (10 litres each) were concentrated through a tangential ultrafiltration system (Sartoflow ® Slice 200 Benchtop Crossflow System, Sartorius AG, Goettingen, Germany), using appropriate membranes (SG Hydrosart 10 kDa) pretreated with 3% Beef Extract (BE) pH 7. Elution and recovery of viruses attached to the membranes employed 3% BE pH 9.5 up to reduce the initial volume to 10-12 ml with pH 7 (Aulicino et al., 1993; Giammanco et al., 2014).

Nucleic acid extraction

The viral genomes (RNA and DNA) were extracted by using commercial kit based on the selective binding of nucleic acids to silica magnetic beads as described by the manufacturer (NucliSENS® miniMAG extraction, bioMe´rieux Italia S.p.A., Rome, Italy). In order to compare the extraction results, for some samples, spin columns were also used, employing the QIAamp® Viral RNA Kit Mini Kit - QIAGEN (RNA), by varying the initial volume of the sample to be extracted (560 µl) and the volume of the eluate (100 µl).

Molecular detection

HAV, GI and GII NoV, RoV and AdV were detected by nucleic acid amplification (end-point and Real Time PCR/RTPCR) and sequencing.

Biomolecular assay amplification kits and references used in this study are shown in the Table 1.

The positive RT-PCR/PCR products obtained were purified using illustra™ GFX™ PCR DNA and Gel Band Purification Kit (GE Healthcare) and sequenced (BMR Genomics, Padova, Italy). The raw forward and reverse ABI files obtained were aligned and assembled into a consensus sequence using MEGA 7 software and sequences submitted to BLAST analysis (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

RESULTS

Table 2 shows the results of the analysis. Of the 72 mollusc samples, 31 (43.0 %) were positive for enteric viruses, showing a high positivity for HAV (18.0%), NoV (16.7% GI, 5.5% GII). AdV was found in 2.8% of the molluscs. No RoV was found in shellfish.

The 13 HAV positive shellfish were genotyped as HAV 1A (one sample) and HAV 1B (12 samples). The 12 GI NoV positive shellfish were classified in six different genotypes for GI NoV (GI.1, GI.2, GI.3 , GI.5, GI.6, GI.8) and three genotypes

for GII NoV (GII.1, GII.2, GII.4). A single genotype was detected for *Adenovirus* (AdV40).

The recovery efficiency, determined on the process control *Mengovirus*, was >1% for all the samples and thus results considered valid according to ISO 15216-2:2013.

Of the 16 water samples, 3 (18.7%) were positive for enteric viruses. Two water samples (12.5%) were positive for GII NoV (GII.2 and GII.4). The superficial freshwater sample were found positive for GII.4 and also for RoV. Data on RoV genotyping are not yet available. None of the other tested enteric viruses were detected in the water samples.

DISCUSSION AND CONCLUSIONS

Viruses are recognized as cause of foodborne and waterborne disease transmitted by the fecal-oral cycle. According to a report by European Food Safety Authority EFSA, enteric viruses were the most commonly detected (20.4%) causative agent in foodborne outbreaks (EFSA, 2015). Among the main foods involved in the transmission of human enteric viruses are molluscs, fruits and vegetables irrigated with wastewater and/or washed with non-potable water, contaminated drinking or recreational water.

Detection of enteric viruses in waters and contamination of aquatic environments have been reported by several studies worldwide (La Rosa et al., 2010a, b; Kamel et al., 2011; Prado et al., 2012; La Rosa et al., 2015; Osuolale & Okoh, 2015). In Sicily an outbreak of gastroenteritis occurred in Agrigento during March 2011, and NoV was identified in water samples from the public water system (Giammanco et al., 2014); a NoV gastroenteritis outbreak at a seaside resort near Taormina (Mascali, Sicily) originated from municipal water distribution system in May 2016 (Giammanco et al., 2018).

Data on food, particularly shellfish and water viral contamination in South of Italy are poor; this study analyzed both matrices for a panel of enteric viruses and provided information on the presence of environmental contamination, chiefly in shellfish production areas, useful to better understand the circulation of viral pathogens able to infect humans in Sicily.

The study found an abundance of viruses in the samples investigated. In the total of the samples, HAV showed the highest percentage of presence (14.8%), followed by GI NoV (13.6%), GII NoV (6.8%). A low percentage was found for AdV (2.8%) and RoV (1.1%). In particular, high positivity for HAV (18.0%) was assessed in bivalve molluscan shellfish samples, investigated as sentinel of marine pollution. No HAV was detected in water samples. Most of water samples (12.5%), object of the present study, were positive for GII NoV.

In the present study NoV was also detected in shellfish (16.7% GI, 5.5% GII). NoV repeatedly cause outbreaks either waterborne or associated with shellfish probably contaminated with human fecal material used as fertilizer (Müller et al., 2016).

The genetic heterogeneity of the viral strains from the positive Sicilian samples were particularly interesting, highlighting the presence of HAV IA and IB, six different genotypes of GI NoV (GI.1, GI.2, GI.3, GI.5, GI.6, GI.8) and of three genotypes of GII NoV (GII.1, GII.2, GII.4). A major single genotype, GII.4, has been associated with the vast majority of NoV-related outbreaks and sporadic cases of acute gastroenteritis (AGE) worldwide (Bok et al., 2009). A single genotype was detected for Adenovirus (AdV40). Data on RoV genotyping are not available.

Our findings clearly demonstrate the presence of human pathogenic virus in aquatic environmentsbe useful as a scientific support for informed risk assessment of foodborne and waterborne diseases and to implement and optimize virological controls in the food chain. For this purpose, an integrated "One Health" surveillance system is clearly needed to obtain molecular data on virus isolated from humans and the environment to rapidly understand possible future viral outbreaks and epidemics.

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Biodiversity Journal, 2019, 10 (4): 445–450 https://doi.org/10.31396/Biodiv.Jour.2019.10.4.445.450
MONOGRAPH

Genetic database development for the characterisation of Sicilian sheep population

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ABSTRACT

The most representative sheep in Sicily are *Belicina*, *Comisana*, *Pinzirita*, *Barbaresca* and the crossbred derived sheep from all this species. In this study, the allelic frequencies of the Sicilian sheep population were investigated. It currently represents the best way to determine the genetic identity and/or family even with limited amounts of sample or when the DNA is degraded. The aim of the study was to provide a reference data bank and to evaluate a microsatellite panel for pedigree analysis as suggested by the International Society for Animal Genetics (ISAG). There are various studies on European sheep, but few datasets were developed on the population of Sicilian sheep. The reference database will include allele frequencies at each locus and will determine genetic parameters for Sicilian ovine species selection. Our results indicated that Hardy Weinberg equilibrium was not always maintained. These results could be explained by a non-random mating. The database is useful to investigate the relationship, the parentage the meat traceability and in disease control programs. The standardized panels of allele frequencies represent a molecular fingerprinting characterizing the subjects with very high definition level and can be useful to control all the livestock. The parentage identification could be important for the veterinary police to investigate the theft or the animal substitutions in the Sicilian farms.

KEY WORDS Ovine; microsatellite; locus; allele.

Received 28.11.2019; accepted 19.12.2019; published online 20.12.2019
Proceedings of the 4th International Congress on Biodiversity "Man, Natural Habitats and Euro-Mediterranean Biodiversity", November 17th-19th, 2017 - Malta

INTRODUCTION

The most representative species of sheep in Sicily are *Belicina*, *Comisana*, *Pinzirita* and *Barbaresca*. The purpose of this study was to investigate the allelic frequencies in the Sicilian sheep population to provide a reference data bank and to evaluate the use of ISAG (International Society for Animal Genetics) through a microsatellite panel for pedigree analysis. Tandem repeats are used as an effective method to track DNA markers in genotyping field. The deriving database can be useful for the traceability of meat, risk assessment and consumer warranty. The microsatellites are employed as genetic markers for their random distribution, the codominant inheritance (Barbarà et al., 2007), discriminative power and possibility of simultaneous analysis. The microsatellite markers can also be used to trace the meat through the production processes (Vázquez et al., 2004), to study the ge-

netic diversity of sheep (Peakall & Smouse, 2012) and to select the animals in breeding programs. Moreover represent the best way to determine the genetic identity and/or family, even with limited amounts of sample or when the DNA is degraded. Commonly the most common approach in ovine breeding systems is the use of multiplesire natural mating based on one or few males. Parentage inaccuracies due to human error getting to wrong animal identification codes can be entered into the herd book (Weller et al., 2004). Significant pedigree record errors seem to be a common problem in sheep that reduces the genetic progress of the populations. Under these scenarios, DNA-based paternity testing provides a powerful tool to carry out precise breeding strategies and improve the overall quality of the flock. DNA Genotyping using marker panels has become the most common procedure for paternity and pedigree testing both in human and livestock species. Many highly polymorphic MST alleles have been studied that are often in the 70–250 bp range. The selection and optimization of a MST panel was successful for parental investigation in randomly chosen animals.

See also other cited bibliography: Barendse et al., 1994; Heyen et al., 1997; Jamieson & Taylor, 1997; Luikart et al., 1999; Diez-Tascon et al., 2000; Farid et al., 2000; Baron et al., 2002; Visscher et al., 2002; Bruford et al., 2003; Senneke et al., 2004; Van Oosterhout et al., 2004a, b; Baumung et al., 2006; Jiménez-Gamero et al., 2006; Glowatzki-Mullis et al., 2007; Kalinowski et al., 2007; Lawson Handley et al., 2007; Ozkan et al., 2009; ISAG, 2010; Carneiro et al., 2010; Dorji et al., 2010; Saberivand et al., 2010; Azhar et al., 2018.

MATERIAL AND METHODS

We tested 10 microsatellite markers recommended by ISAG on 452 Sicilian sheep. The microsatellite loci were employed in two homogeneous multiplex group of loci, we use ten ISAG loci for present study: OarFCB011, INRA0063, HSC, OarCP0049, OarFCB0304, CSRD0247, OarFCB020, D5S2, SPS0113, INRA005. Whole blood samples were taken from the Sicilian typical half-breed, species representative of the Sicilian population. Genomic DNA was extracted and purified using a commercial kit (Ezna WVR). Two different PCR test were employed to investigate a panel of ten microsatellites. DNA targets were amplified in a 6-plex and 4-plex PCRs system respectively as follows: 12.5 µl Type-it 2X master mix (Qiagen), 2.5 µl Primer mix (2 µM for each primer), 20 ng DNA. PCRs were carried out using a thermocycler (9700 Applied Biosystems, San Diego, CA, USA). Multiplex-PCR products were analyzed using an ABI PRISM 3130 Genetic Analyzer (Applied Biosystems). Genotypic profiles

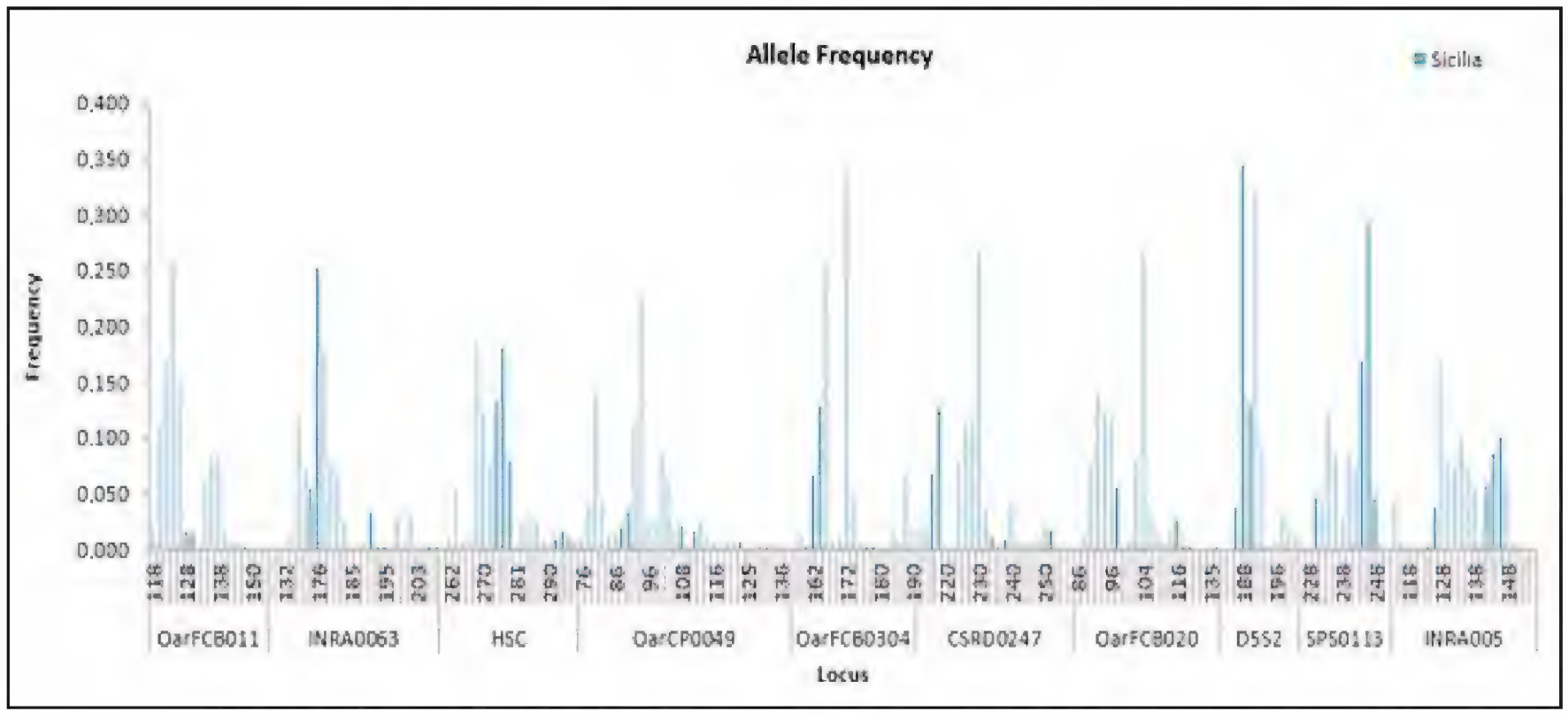

Figure 1. Allele frequency observed at each locus for every examined samples. (GenAlEx v6.5 software).

Locus	N	NA	NE	NE/NA	MAF	HO	HE	Fis	PIC	PE	PI
OarFCB011	**452**	**22**	**8.42**	**0.383**	**0.187**	**0.771**	**0.881**	**0.007**	**0.853**	**0.80**	**0.033**
INRA0063	**452**	**31**	**13.40**	**0.433**	**0.138**	**0.772**	**0.925**	**0.090**	**0.914**	**0.85**	**0.023**
HSC	**452**	**20**	**9.75**	**0.488**	**0.188**	**0.783**	**0.897**	**0.024**	**0.876**	**0.81**	**0.022**
OarCP0049	**452**	**36**	**12.51**	**0.347**	**0.161**	**0.763**	**0.922**	**0.091**	**0.907**	**0.89**	**0.014**
OarFCB0304	**452**	**28**	**5.54**	**0.191**	**0.345**	**0.651**	**0.819**	**0.024**	**0.754**	**0.83**	**0.063**
CSRD0247	**452**	**37**	**9.68**	**0.262**	**0.197**	**0.804**	**0.897**	**0.001**	**0.875**	**0.87**	**0.024**
OarFCB020	**452**	**24**	**8.79**	**0.366**	**0.187**	**0.758**	**0.886**	**0.030**	**0.860**	**0.82**	**0.028**
D5S2	**452**	**13**	**4.14**	**0.319**	**0.329**	**0.614**	**0.759**	**-0.050**	**0.642**	**0.68**	**0.095**
SPS0113	**452**	**18**	**7.11**	**0.395**	**0.235**	**0.642**	**0.859**	**0.112**	**0.820**	**0.77**	**0.034**
INRA005	**452**	**30**	**12.22**	**0.407**	**0.130**	**0.681**	**0.918**	**0.176**	**0.905**	**0.82**	**0.013**
Mean		**25.9**	**9.157**	**0.359**	**0.209**	**0.723**	**0.876**	**0.051**	**0.841**	**0.81**	**0.034**

Table 2. Genetic parameters in Sicilian sheep population. NA = number of alleles; NE = number of effective alleles; MAF = minor allele frequency; HO = observed heterozygosity; HE = expected heterozygosity; Fis = inbreeding coefficient; PIC = polymorphic information content; PE = probability of exclusion; PI = probability of identity.

were read and analyzed by GeneMapper software v4.0 (Applied Biosystems). Statistical analysis of data generated from the 10 markers that were analyzed was performed using GenAlEx (Peakall & Smouse, 2012), PowerMarker (Liu & Muse, 2005), and Micro-Checker (Van Oosterhout et al., 2004) softwares. All these are a useful set of bioinformatic tools specific for genetic populations analysis implementing various data management algorithms. The number of effective alleles (N_E), allele number (N_A), allele frequency, observed and expected heterozygosities (H_O and H_E, respectively), probability of Identity (P_{ID}), defined as the probability that two individuals drawn at random from a population will have the same genotype at multiple loci, probability of exclusion of a locus (P_E), and the devia-

tion probability from the Hardy-Weinberg equilibrium (HWE) were obtained using the GenAlEx v6.5 software. The expected homozygosity (HomE), observed homozygosity (HomO), homozygosity excess, evidence for null allele, evidence for large allele dropout, and evidence for scoring error due to stuttering were obtained using the Micro-Checker v2.2 software. The polymorphism information content (PIC), inbreeding coefficient, genotype number (NG), major allele frequency (MAF), and major genotype frequency (MGF) were investigated using the PowerMarker v3.25 software.

RESULTS

In total, 452 related and unrelated sheep were genotyped, the average observed heterozygosity was lower than the expected value (0.701 vs 0.852). The exact test for Hardy-Weinberg proportion, allele number and inbreeding coefficient were calculated. These results could be explained by a non-random mating studies on a larger number of samples. The polymorphic information content (PIC) calculated according Botstein et al. (1980) ranged from 0.642 for locus D5S2 to 0.914 for locus INRA0063. PIC is a parameter that indicates the degree of marker informativeness describing genotypic variation in single base pair or in larger sequence repeats. The PIC value will be almost zero if there is no allelic variation. All locus were informative (PIC > 0.5) (Table 1), with a mean PIC of 0.841. The heterozygosity expected H_E and observed H_O, as measures of genetic diversity at a single locus, are shown in Table 1. In all cases, H_O was lower than H_E.

DISCUSSION AND CONCLUSIONS

To establish a livestock conservation program it is fundamental the genetic characterization of the entire population under study. For different breeds must have taken into account also phenotypic differences (morphology, milk production, disease resistance etc) and information about provenience of samples. A database of frequencies for the different alleles of known microsatellite markers it's important to help researcher in studying the phylogeny of one or more populations, to discover patterns of relationships among different groups or associate the genetic markers with important productive characteristics. Analysis of different samples of sheep resulted in a set of genotype profiles of the most representative ovine populations from Sicily. The principal statistics parameter of sheep population were obtained elaborating microsatellites alleles frequencies through a set of statistical analysis tools in particular Genalex and PowerMarker software and Micro-Checker. The final data show a significant deficiency in the H_O value compared to the H_E value. This deviation from HWE can be caused by inbreeding, assortative mating or Wahlund effect, due to a fragmentation of the original population into subpopulations. But loss of heterozygosity can also include genotyping errors due to nonamplified alleles (null alleles) caused by mutations in primer binding site (Pemberton et al., 1995), short allele dominance (large allele dropout) and the scoring of stutter peaks dropout. Finally, we observed an homozygote excess in all loci compared to the expected value. Five loci (OarFCB0304, CSRD0247, D5S2, SPS0113, INRA005) showed a strong evidence for scoring error due to stuttering, and none of these loci had large allele dropout. It is not clear whether the homozygote excess is due to null alleles or if it really reflects the genotypes of the Sicilian sheep population.

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Biodiversity Journal, 2019, 10 (4): 451–456 https://doi.org/10.31396/Biodiv.Jour.2019.10.4.451.456
MONOGRAPH

Creation of a pollen database for Mediterranean flowering plants

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ABSTRACT

Palynology is the science that studies pollen grains (size, morphology, structure, function, ornamentation, physical and chemical properties), the carriers that transport the male gametes to the pistil (more precisely to the stigma) allowing the fertilization of the eggs. In seed plants pollens represent an extra generation (haploid generation), the widely reduced male gametophyte. During the pollen release phase, the pollen grains separate completely from the plant (diploid generation, or sporophyte) in an attempt to reach the female flower to allow the release of genetic material and, therefore, the fertilization of the egg. Pollens possess many varieties of shapes, sizes, designs, ornamentations, openings with variable shapes and numbers that can be observed by optical microscopy and that have a high systematic value. Each botanical species has pollens with unique characteristics that allow their identification. Palynology is widely used as an extremely important tool in various types of studies and investigations, such as paleobotany, forensic investigations, melissopalynology, studies on the biodiversity of precise geographical areas, identification of cases of introduction of non-native species and identification of hybridization between species. For these reasons the creation of a pollen database could be a particularly efficient and useful tool.

KEY WORDS Palynology; biodiversity; pollen database.

Received 12.10.2019; accepted 29.11.2019; published online 20.12.2019
Proceedings of the 4th International Congress on Biodiversity "Man, Natural Habitats and Euro-Mediterranean Biodiversity", November 17th-19th, 2017 - Malta

INTRODUCTION

One of the consequences of the decline of biodiversity is the reduction in the quantity, quality and diversity of pollen, which is a fundamental food source for bees, dependent on environmental changes. Bees have also been affected with unprecedented worldwide declines. Today we are witnessing a true 'eco-drama'.

Biodiversity is the whole and the variety of all living things, plant and animal forms, present in the ecosystems of the planet. The concept of biodiversity is closely linked to the life of each living form: greater biodiversity provides a greater chance of survival. Even for pollen, diversity of pollen is key.

For the purpose of the pollen database, various pollen species were investigated by performing microscopic analysis (Erdtman, 1943; Moore et al., 1991).

Pollen structure

Generally mature pollen grains are circular or elliptical, have variable dimensions, and are cov-

ered by a layer of lipids and carotenoids and other substances which facilitate their adhesion on the stigma surface (pollenkitt) (Fig. 1).

Pollen has sizes ranging from 10 μm to 100 μm. This variety of dimensions is considered a diagnostic character. It may also depend on the degree of hydration or dehydration of the pollen or the method of preparation.

The use of the following categories could be helpful: very small (<10 μm), small (10–25 μm), medium (26–50 μm), large (51–100 μm) and very large (> 100 μm).

Pollen grains have openings from which the genetic material exits to fertilize the eggs: colpus (elongated) and pores (circular). A combination of porus and colpus is termed a colporus (Knox & McConchie, 1986; Blackmore & Crane, 1998; Banks, 2003).

The presence of colpus, pores (or both), their number, size and disposition is another character to make a classification of the different pollen forms, referable to specific botanical species.

The pollen wall

The pollen has a protective wall (sporoderm) composed of two layers: intine and exine (Blackmore et al., 2007; Rowley & Skvarla, 2000). The intine is constituted by polysaccharides such as cellulose, pectine and hemicellulose, the exine is constituted by sporopollenin. Commonly, the pollen wall in apertural regions is characterized by the reduction of exinous structures or by a deviant exine, and a thick, often bilayered intine.

Exin can be divided into: endexine and ektexine (Bor, 1979).

The ektexine consists of a basal foot layer, an infratectum and a tectum, the endexine is a mainly unstructured, single layer (Fig. 2).

In the regions of openings the pollen wall is characterized by a different exine construction.

The terms sexine for the outer, structured, and nexine for the inner, unstructured exine layer are widely used in light microscopy, but do not fully correspond to ekt- and endexine, respectively.

The angiosperm pollen wall

In angiosperms, ektexin is generally made up of tectum, infra tectum and the foot layer (Knox, 1984). The tectum, more or less continuous, can be covered by supertectal elements. The infratectum can be columellate or granular. The foot layer can be continuous, not continuous or totally absent. Endexine can be continuous, not continuous, compact or spongy, present only in the openings or absent (Erdtman, 1952; Doyle, 2005).

The gymnosperm pollen wall

The pollen wall of the gymnosperms is different from that of the angiosperms in two characters: 1. the endexine is always lamellatE in the mature pollen; 2. the infratectum can be granular or alveolate but it is never columellate (Gullvåg, 1966; Van Campo, 1971).

The main stratification (ektexine, endexine and

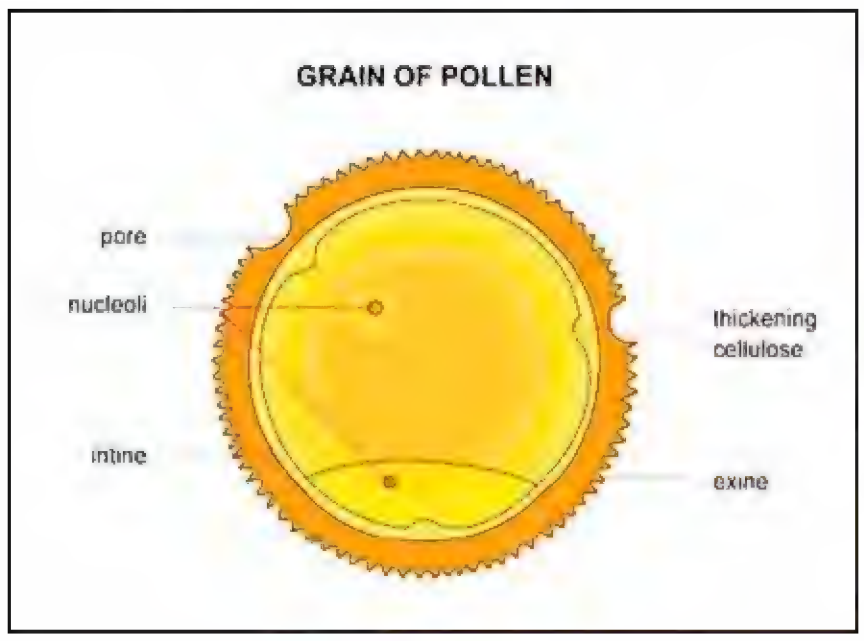


Figure 1. Pollen structure.

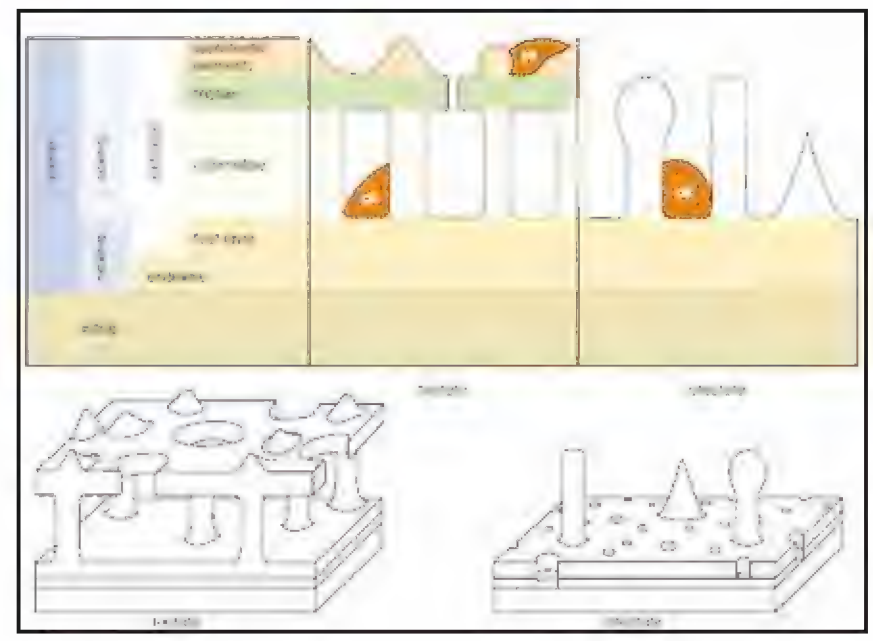


Figure 2. Pollen wall stratification.

intine) is the same in gymnosperms and angiosperms.

Dispersal units

Mature pollen is shed in dispersal units. The post-meiotic products either remain permanently united or become partly or usually completely disintegrated.

In the latter case the dispersal unit is a single pollen grain, a monad; if the post-meiotic products remain united, dyads (a rare combination), tetrads or polyads are the result.

Place of collection	Species collected	Honey species
Piana degli Albanesi	4	2
Botanical Garden of Palermo	3	0
Ficuzza wood	7	5
Mezzojuso	6	3

Table 1. Places of collection and species collected.

MATERIAL AND METHODS

The presence of structures and ornamentations on the outer portion of the exine, allows to attribute a certain pollen morphology to a well-defined botanical species.

Also important for recognition are the openings that may be present in pollen grains, having very variable shapes and sizes (Pozhidaev, 2000).

Pollens without openings are called inaperturate, while pollens with openings are called aperturate.

Depending on the morphology, the openings are classified as colpus and pores. Pollens that have pores are called porates, pollens that present colpus are called colpates (Erdtman, 1945, 1957; Iversen & Troels-Smith, 1950; Jerković et al., 2010).

Also, the number and position of colpus and pores is useful for recognition as it varies from species to species.

The first step of the present work was the collection of the flowers, choosing those with pollen-filled anthers.

The collection was carried out in various sites near Palermo (Sicily, Italy), such as the Botanical Garden of Palermo, the Ficuzza wood, Mezzojuso and Piana degli Albanesi.

Since this study is also preliminary to a second work in which we deal with melissopalynology and variability of pollen representation in unifloral honeys, we have given much importance to those floral species used for honey production (Table 1).

The second step is the palynological analysis, which consists in the observation, by optical microscope, of the pollen collected directly from the anthers of the flowers.

The pollen is collected by washing the anthers with ethyl ether, $(C_2H_5)_2O$, and then placed on a microscope slide to proceed with microscopic observation.

Observation by optical microscope can be carried out naturally or with the use of a dye such as fuchsin, that enhances the morphological characteristics the exine. This gives the pollen a magenta color with different intensity, depending on the permeability of the pollen and the concentration of the dye. Glycerine gelatin was also used to fix the pollen to the glass slide. Once the slides have been prepared with the collected pollen, we passed to the observation with the optical microscope. Based on the size of the pollen grains, different magnifications (10x, 40x and 100x) were used.

The two different observation methods, with fuchsin and without fuchsin, allow us to study the various pollen morphologies that are photographed, examined, cataloged and included in a palynological database that can be consulted for various purposes (botanical, paleobotanical, forensic, melissopalynology, biodiversity) (Figs. 3–8).

The observation without fuchsin highlights the shape, size and color of the pollen; the use of fuchsin allows us to study in more detail the structure of the exine with its various ornamentations and structures. The observation without fuchsin allows to study other natural characteristics of pollen, such as their color.

RESULTS AND CONCLUSIONS

In order to make the microscopic analysis more

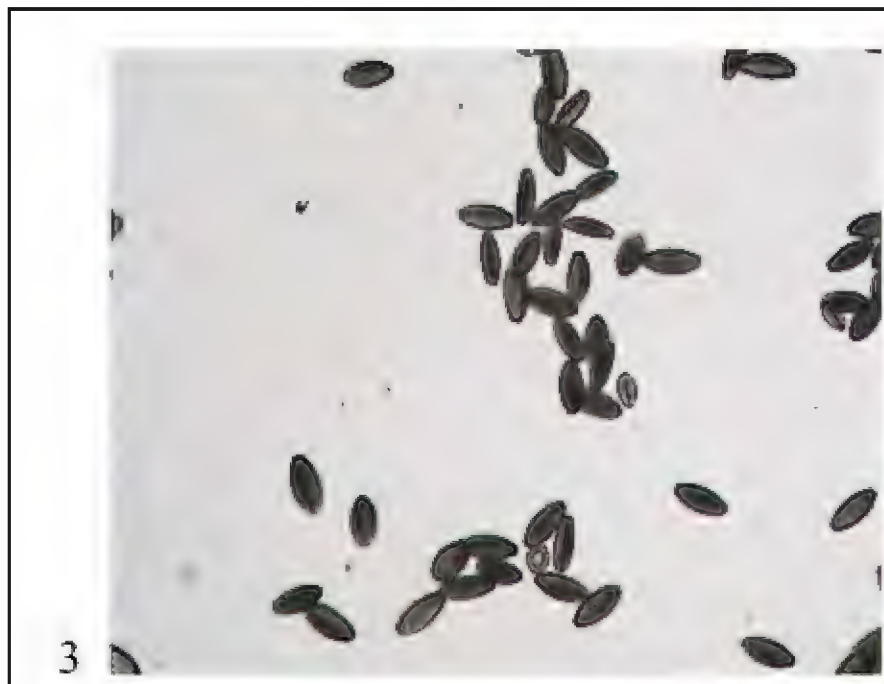
accurate, observations were also made using fuchsin, a dye that enhances the morphological characteristics of the pollen, especially the exine, the outer layer of the pollen grain.

Through natural observation and with fuchsin, we observed, studied and cataloged the various pollen morphologies belonging to different floral species present in the Mediterranean area, such as Rosemary, Sulla, Dandelion, Asphodel, Thistle, *Ailanthus*, Almond, Chestnut, Carob tree, etc.

The data collected through our observations were compared with data from other palynological encyclopedias and, then, we proceeded to create a palynological database of the main floral species of the Mediterranean. The steady development and updating of a palynological database could be very important for the study of biodiversity, because it would provide an additional tool to identify, for example, cases of introduction of non-native species, cases of speciation or hybridization.

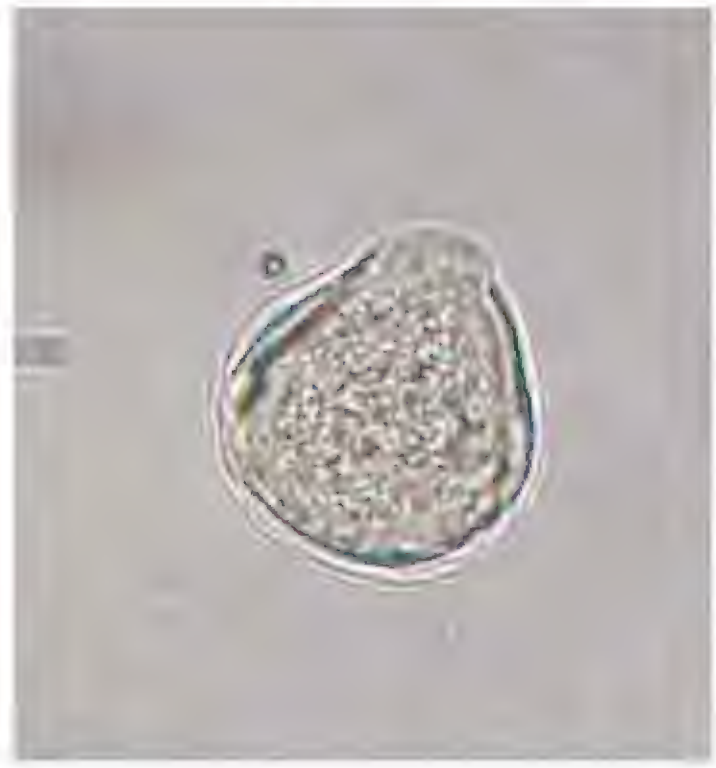
Species	Pollen unit	Pollen class	Aperture number	Aperture type	Size
Ailanthus altissima	monad	colporate	3	colporus	26 - 60 µm
Asphodelina lutea	monad	sulcate	1	sulcus	51 - 100 µm
Asphodelus ramosus	monad	sulcate	1	sulcus	51 - 100 µm
Castanea sativa	monad	colporate	3	colporus	10 - 25 µm
Ceratonia siliqua	monad	colporate	4	colporus	26 - 50 µm
Citrus aurantium	monad	colporate	4	colporus	26 - 50 µm
Eucalyptus camaldulensis	monad	colporate	3	colporus	10 - 25 µm
Euphorbia dendroides	monad	colporate	3	colporus	26 - 50 µm
Ferula communis	monad	colporate	3	colporus	26 - 50 µm
Galactites tomentosa	monad	colporate	3	colporus	26 - 50 µm
Hedysarum coronarium	monad	colpate	3	colpus	10 - 25 µm
Malva neglecta	monad	colporate, inaperturate	>6	porus	51 - 100 µm
Oxalis pes-caprae	monad	colpate	3	colpus	26 - 50 µm
Pinus pinea	monad	saccate	1	leptoma	50 - 100 µm
Pyrus pyraster	monad	colporate	3	colporus	26 - 50 µm
Prunus dulcis	monad	colporate	3	colporus	26 - 50 µm
Quercus ilex	monad	colpate	3	colpus	26 - 50 µm
Rosmarinus officinalis	monad	colpate	6	colpus	26 - 50 µm
Rubus ulmifolius	monad	colporate	3	colporus	10 - 25 µm
Taraxacum officinale	monad	iophate	3	colporus	26 - 50 µm

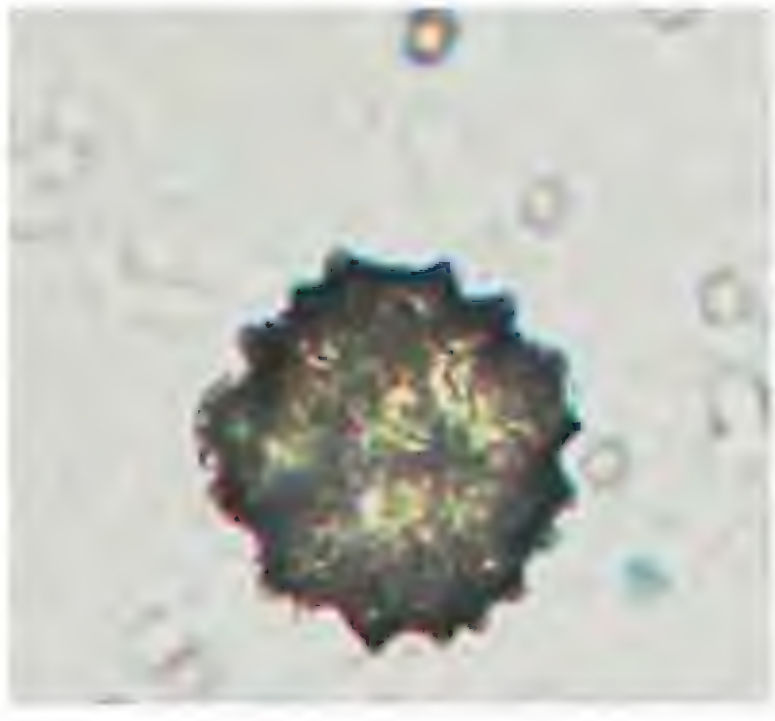
Table 2. Characteristics of analyzed pollens.


3


4


5


6


7

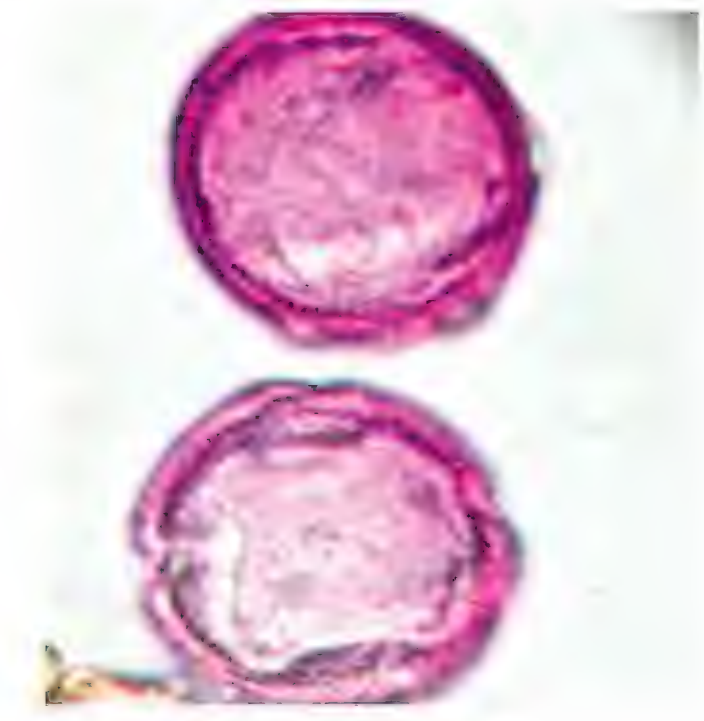

8

Figure 3. Pollen of *Pyrus pyraster* (10x). Figure 4. Pollen of *Castanea sativa* (40x). Figure 5. Pollen of *Pinus pinea* (40x). Figure 6. Pollen of *Ailanthus altissima* (40x). Figure 7. Pollen of *Galactites tomentosa* (40x). Figure 8. Pollen of *Citrus aurantium* with fuchsin (40x).

This study was also necessary for another more complex work, which consists of melissopalynological analysis of the honeys both through the use of optical microscopy, for the recognition of pollen present in honey through comparison with the data present in our database, and through the use of Molecular Biology techniques, such as DNA extraction, PCR and real-time PCR, which will allow a more precise analysis (Loveaux et al., 1978; Simonetti et al., 1989; Persano Oddo et al., 1995; Schievano et al., 2013; Mannina et al., 2015).

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Biodiversity Journal, 2019, 10 (4): 457–461 https://doi.org/10.31396/Biodiv.Jour.2019.10.4.457.461
MONOGRAPH

Molecular barcoding applied to the Mediterranean turtles biological matrices (Reptilia Cheloniidae)

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ABSTRACT

Chelonia mydas (Linnaeus, 1758) together with *Caretta caretta* (Linnaeus, 1758) is the most representative Cheloniidae species in the Mediterranean basin. Currently, at the National Reference Centre in the "Istituto Zooprofilattico" of Sicily (Italy), damaged subjects are rehabilitated before they are released again. Clinical, physiological and molecular parameters were collected from each subject. We analysed 46 turtles which samples were collected. Species specific Cytochrome oxidase I sequences for the identification of marine turtle species were obtained. Barcoding is a new tool of classical taxonomy that allows the characterisation of living species and the differentiation of very morphologically similar species. It is a practical tool that can be used in cases of damaged samples and is also useful for taxonomical characterisation of specimen at immature development stages. In our region, in the centre of the Mediterranean area, we represent a reference centre for injured animals both stranded on the beach and captured in offshore.Turtles caught in fishing lines generally retain the fishing hooks in their throat or oesophagus, as visible by X-ray investigations. After the cure and samples collection, the animals are released into the sea. The polymorphisms could be related to the geographical distance of the turtles following different routes during their life. The large-scale sequencing of a single or few genes in taxonomic studies, denominated by species barcoding, aims at offering a practical method for species identification, as well as for providing insights into the evolutionary diversification of life.

KEY WORDS

Turtles; Mediterranean Sea; molecular barcoding; speciation.

Received 28.11.2019; accepted 19.12.2019; published online 20.12.2019
Proceedings of the 4th International Congress on Biodiversity "Man, Natural Habitats and Euro-Mediterranean Biodiversity", November 17th-19th, 2017 - Malta

INTRODUCTION

Three species of sea turtles lives in Mediterranean Sea: the leatherback turtle, *Dermochelys coriacea* (Vandelli, 1761), the green turtle, *Chelonia mydas* (Linnaeus, 1758), and the loggerhead turtle, *Caretta caretta* (Linnaeus, 1758). Two other species of sea turtles have been occasionally reported in the Mediterranean Sea: the hawksbill sea turtle, *Eretmochelys imbricata* (Linnaeus, 1776), and the Kemp's ridley sea turtle, also called the Atlantic ridley sea turtle, *Lepidochelys kempii* (Garman, 1880) (Casale & Margaritoulis, 2010). They are considered endangered, at regional and global level, and therefore it is protected by international laws and by numerous conventions.

Dermochelis coriacea is a Vulnerable species following IUCN (Wallace et al., 2013), *Chelonia*

mydas is Endangered (Seminoff, 2004), and *Caretta caretta* is Vulnerable (Casale & Tucker, 2017). Only *Chelonia mydas* and *Caretta caretta* breed in the Mediterranean basin and the nesting areas are concentrated in the eastern half of the Mediterranean Sea. These sea turtles are found, also, with particular frequency in some rest and feeding areas (like northern Adriatic Sea, Ionian Sea, Tunisia and Libya and Spanish coasts).

Chelonia mydas frequents the eastern Mediterranean with the nesting sites that are principally in Turkey, Cyprus, and Syria. Foranging areas are also in Greece and Libya and green turtles are found occasionally in the Adriatic Sea, Tunisia and other areas of the western Mediterrannean Sea.

Caretta caretta known also as common turtle, is the most widespread species and most representative Cheloniidae family species in the Mediterranean Sea, followed by *Chelonia mydas* (Dutton, 1996; Carreras et al., 2007; Casale & Margaritoulis, 2010; Naro-Maciel et al., 2010). *Caretta caretta* is the only species of sea turtle nesting along the Italian coast. In the past, loggerhead sea turtles nesting was a regular phenomenon and relatively widespread along the coasts of the southern Italy, but over the last years, few cases of nesting have been recorded on the islands and coasts of Sicily, Sardinia, along the Ionian coast of Puglia and those of Basilicata and Calabria. However, nests are now considered sporadic or occasional, except for the Lampedusa and Linosa Islands. In the Italian coasts, their strandings are strongly influenced by the impact of massive fishing, the alteration of marine and coastal habitats and climate changes (Tamura et al., 2004). Currently, at the National Reference Centre for sea turtles located in the Istituto Zooprofilattico Sperimentale of Sicily (IZS Sicily, Italy), damaged subjects rescued along the Sicilian coasts are hospitalized, cured and freed again in the marine environment. Many exams are conducted in each subject including molecular parameters. Currently, there is a new tool to help track this highly migratory and endangered group of marine animals: DNA barcodes. DNA barcodes are short genetic sequences that efficiently distinguish species from each other, even if the samples from which the DNA is extracted are minute or degraded. DNA barcodes are relatively short segments of mitochondrial DNA. A region of the COI, or cox1 gene (cytochrome c oxidase subunit 1) has been agreed upon by researchers as appropriate for barcoding, given that it is both highly variable and very specific. This portion of the genome mutates quickly enough to distinguish many closely related species but also slowly enough so that individuals within a species may have similar barcodes. The aim of the present study was to obtain species-specific COI barcode tags that can be used for identifying individually the marine turtle species studied. Indeed, the large-scale sequencing of a single or few genes in taxonomic studies, denominated the Barcode initiative, aims at representing a practical method for species identification, as well as for providing insights into the evolutionary diversification of life (Honda et al., 2002; Hudson & Buhlmann, 2002; Hebert et al., 2003).

MATERIAL AND METHODS

Samples were collected from muscle tissue or blood of both dead and live turtles rescued. DNA was extracted with EZNA Tissue DNA kit (WVR) and spectroscopically quantized. The primers used for Cyt-b targeted PCR were 5'CTCACCAGACATCTCCATAGC-3' as forward and 5' GGGTTGTTTGAGCCTGTTTCGTG-3' as reverse amplifying a 545 bp long fragment. PCR program was optimised as follow: 94°C for 8 minutes; 40 cycles of the repetitions 94°C for 50 seconds, 55°C for 50 seconds, 72°C for 1 minutes; finally 72°C for 7 minutes. PCR products were visualized on a 1%

Number of Subjects	BLAST code	SPECIES
52	AY678314.1	*Caretta caretta*
2	AF385671.1	*Caretta caretta*
51	JX454984.1	*Caretta caretta*
26	KP256531.1	*Caretta caretta*
8	FR694649.1	*Caretta caretta*

Table1. BLAST code relative to the identified subjets by mtDNA sequencing.

agarose gel, purified and employed in sequencing analysis by Big Dye sequencing kit, (Applied Biosystems), according to the manufacturer instructions. After purification, the products were analyzed on Abi Prism 3130 Genetic Analyzer (Thermo). ClustalW2 software was employed for Gene Bank sequence data comparison and sequence multiple alignment for polymorphism detection. Data obtained in this work was used to create a barcode database for the Cyt-b gene sequence characterizing each turtle cured at the National Reference Centre for sea turtles (Istituto Zooprofilattico Sicilia - Sicily, Palermo).

RESULTS

Out of 110 animals studied, all the sequences analyzed by Clustal W2 software ruled out a 99-100% sequence similarity to *Caretta caretta* species. Multiple alignment revealed a certain percentage of polymorphic SNPs, which could mark genetic variability through the various subject of the same species. The data here obtained allow to develop a database for the sequences barcoding. From the search for polymorphisms there is the possibility of identifying, on the one hand the species, on the other the small differences that may have a phylo-

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1           GCATCTACCTCCACATCGGACGAGGAATCTACTACGGTTCCTATCTATACAAAGAAAC 111
8           GCATCTACCTCCACATCGGACGAGGAATCTACTACGGTTCCTATCTATACAAAGAAAC 102
12          GCATCTACCTCCACATCGGACGAGGAATCTACTACGGTTCCTATCTATACAAAGAAAC 103
14          GCATCTACCTCCACATCGGACGAGGAATCTACTACGGTTCCTATCTATACAAAGAAAC 103
19          GCATCTACCTCCACATCGGACGAGGAATCTACTACGGTTCCTATCTATACAAAGAAAC 104
6           GCATCTACCTCCACATCGGACGAGGAATCTACTACGGTTCCTATCTATACAAAGAAAC 112
20          GCATCTACCTCCACATCGGACGAGGAATCTACTACGGTTCCTATCTATACAAAGAAAC 105
16          GCATCTACCTCCACATCGGACGAGGAATCTACTACGGTTCCTATCTATACAAAGAAAC 103
2           GCATCTACCTCCACATCGGACGAGGAATCTACTACGGTTCCTATCTATACAAAGAAAC 112
4           GCATCTACCTCCACATCGGACGAGGAATCTACTACGGTTCCTATCTATACAAAGAAAC 108
3           GCATCTACCTCCACATCGGACGAGGAATCTACTACGGTTCCTATCTATACAAAGAAAC 111
18          GCATCTACCTCCACATCGGACGAGGAATCTACTACGGTTCCTATCTATACAAAGAAAC 111
10          GCATCTACCTCCACATCGGACGAGGAATCTACTACGGTTCCTATCTATACAAAGAAAC 111
11          GCATCTACCTCCACATCGGACGAGGAATCTACTACGGTTCCTACCTATACAAAGAAAC 115
7           GCATCTACCTCCACATCGGACGAGGAATCTACTACGGTTCCTATCTATACAAAGAAAC 112
9           GCATCTACCTCCACATCGGACGAGGAATCTACTACGGTTCCTATCTATACAAAGAAAC 115
15          GCATCTACCTCCACATCGGACGAGGAATCTACTACGGTTCCTATCTATACAAAGAAAC 114
23          GCATCTACCTCCACATCGGACGAGGAATCTACTACGGTTCCTATCTATACAAAGAAAC 113
22          GCATCTACCTCCACATCGGACGAGGAATCTACTACGGTTCCTATCTATACAAAGAAAC 111
            ******************************************* **************

1           CTGAAATACCGGAATCATCCTCTTACTACTAGTAATAGCCACCGCATTCGTAGGCTACGT 171
8           CTGAAATACCGGAATCATCCTCTTACTACTAGTAATAGCCACCGCATTCGTAGGCTACGT 162
12          CTGAAATACCGGAATCATCCTCTTACTACTAGTAATAGCCACCGCATTCGTAGGCTACGT 163
14          CTGAAATACCGGAATCATCCTCTTACTACTAGTAATAGCCACCGCATTCGTAGGCTACGT 163
19          CTGAAATACCGGAATCATCCTCTTACTACTAGTAATAGCCACCGCATTCGTAGGCTACGT 164
6           CTGAAATACCGGAATCATCCTCTTACTACTAGTAATAGCCACCGCATTCGTAGGCTACGT 172
20          CTGAAATACCGGAATCATCCTCTTACTACTAGTAATAGCCACCGCATTCGTAGGCTACGT 165
16          CTGAAATACCGGAATCATCCTCTTACTACTAGTAATAGCCACCGCATTCGTAGGCTACGT 163
2           CTGAAATACCGGAATCATCCTCTTACTACTAGTAATAGCCACCGCATTCGTAGGCTACGT 172
4           CTGAAATACCGGAATCATCCTCTTACTACTAGTAATAGCCACCGCATTCGTAGGCTACGT 168
3           CTGAAATACCGGAATCATCCTCTTACTACTAGTAATAGCCACCGCATTCGTAGGCTACGT 171
18          CTGAAATACCGGAATCATCCTCTTACTACTAGTAATAGCCACCGCATTCGTAGGCTACGT 171
10          CTGAAATACCGGAATCATCCTCTTACTACTAGTAATAGCCACCGCATTCGTAGGCTACGT 171
11          CTGAAATACCGGAATCATCCTCTTACTACTAGTAATAGCCACCGCATTCGTAGGCTACGT 175
7           CTGAAATACCGGAATCATCCTCTTACTACTAGTAATAGCCACCGCATTCGTAGGCTACGT 172
9           CTGAAATACCGGAATCATCCTCTTACTACTAGTAATAGCCACCGCATTCGTAGGCTACGT 175
15          CTGAAATACCGGAATCATCCTCTTACTACTAGTAATAGCCACCGCATTCGTAGGCTACGT 174
23          CTGAAATACCGGAATCATCCTCTTACTACTAGTAATAGCCACCGCATTCGTAGGCTACGT 163
22          CTGAAATACCGGAATCATCCTCTTACTACTAGTAATAGCCACCGCATTCGTAGGCTACGT 171
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Figure 1. Multiple alignment of sequences of some turtles examined. No significative polymorphic bases were found.

genetic meaning useful for understanding the mechanisms of evolution and species. Moreover the analysis of the incidence of these polymorphisms can give indication on the mutational effects, on possible linkage maps, on the frequency of SNPs in the various subjects examined. However, from the sequence analysis among all the examined animals, no SNPs were found.

DISCUSSION AND CONCLUSION

As previously described in other animal species or in other geographical areas, the barcode is here proposed as a new tool of classical taxonomy which allows the characterization of living species as well as the differentiation of the individuals from the same origin and the others from a similar population within the same species. This practical and economical tool can also be used in cases of damaged samples and of immature state of development of the individual investigated including lost nests. The polymorphisms found in our results could be related to geographical distance of the turtles, which follow different routes during their, life and the Mediterranean Sea for the warm temperature during their reproductive activities. However, this preliminary data should be amplified in a larger population to better understand the migration faced by these animals into the Mediterranean basin as well as in other geographic areas of the world (Tamura et al., 2004). The potential for DNA barcoding applications is significant and often means that the species identity or geographic origin of a product is difficult to ascertain using conventional means. Barcoding items collected by wildlife management permit to track international trade in wildlife products. In addition, protected animals trapped can be identified through DNA barcoding. To assist in these efforts, barcode sequences from this study have been supplied to the Barcode of Life database and GenBank (Kocher et al., 1989), so that the data are freely available. DNA barcoding promises to be a powerful tool for species identification (Stoeckle, 2003; Stuart & Parham, 2004; Vargas et al., 2009), and other conservation genetic applications in marine turtles, which are unique on the evolutionary tree of turtles for occupying the marine realm, and widely known for their extensive migrations. Species identification, one of the main goals of the DNA barcoding initiative, was successfully carried out using their COI sequences (Naro-Maciel et al., 2010). Distance based analysis of COI sequences consistently grouped members of the same species, although a complete sample was necessary for correct assignment using phenetic methods. There was no convincing evidence of cryptic species revealed in this research, a result that is concordant with many other genetic studies of marine turtles. In addition, the barcodes provided insight into population structure and history. However, hybridization is an important source of error for analyses relying solely on a mitochondrial marker, including in this group that is known to hybridize despite ancient separations.

Cytochrome c oxidase subunit I barcodes were obtained for each marine turtles, using discrete characters, more consistent with classical taxonomy than distance based methods. Importantly, the character based approach was reliable, no species diagnoses could be made if the query sequences did not contain the relevant diagnostic characters.

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Biodiversity Journal, 2019, 10 (4): 463–464 https://doi.org/10.31396/Biodiv.Jour.2019.10.4.463.464
MONOGRAPH

Investigation on the presence of Dioxins in the Sicilian Sheep's milk

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ABSTRACT Our studies were conducted in 2016 and several sicilian sheep farms were involved. The goal of this research was to evaluate the possible use of sheep as a "sentinel" body to be used as a biological indicator for environmental biomonitoring of dioxins. For the determination of dioxins in sheep's milk was used a method on high-resolution gas chromatography along with a high resolution mass spectrometry (HRGC/HRMS) in order to evaluate the existence of dioxins with concentrations exceeding the limits imposed by the EC Regulation 1881/2006. A total of 200 samples were analyzed, of which 14 samples exceeded the limits imposed by the EC Regulation 1881/2006: 3.0 pg/g fat sum of dioxins (WHO.PCDD/F-TEQ). It is conceivable that the proximity to high anthropization areas and industrialization, may have a positive influence on the propagation of dioxins in the environment having been detected values exceeding the allowed limit. The samples with low dioxin concentrations were analyzed, with values not exceeding the prescribed limit.

KEY WORDS Dioxins; milk; PCDD; GC/MS.

Received 22.11.2019; accepted 10.12.2019; published online 20.12.2019
Proceedings of the 4th International Congress on Biodiversity "Man, Natural Habitats and Euro-Mediterranean Biodiversity", November 17th-19th, 2017 - Malta

INTRODUCTION

Dioxins are present in the soil as trace impurities coming from herbicides and chlorophenols. They can originate from photochemical and thermal reactions in fly ash and other incineration products. The stability in chemical structure and in the environment of some dioxins coupled with their potential to accumulate in fat is confirmed in their detection into global ecosystem (Eisler, 1986).

MATERIAL AND METHODS

The sheep breeds of the farms under investigation come from crosses between Cosimana, Maltese, Derivate of Syria, Argentine Etna, Girgentana and Messina Indigenous. They were bred year-round semi-wild state. Their nutrition consists mainly of fodder provided by the adjacent pasture-grazing areas integrated, in the least, with dried fodder (lager hay). The first step in the determination was the extraction of the fat fraction from the milk sample. The analytical technique used is the accelerated extraction with the solvent (ASE Thermo Fisher Scientific). Approximately 10 mL of sample was added 200 μL of internal standard (PCB 155) and diatomaceous earth. The extraction solvent is a mixture of hexane/ether 30:70 (V/V). The second step is to purify the fat extract through the passage

Compound Name	Quant /Qual	Precursor Ion [Da]	Product Ion [Da]	Collision Energy [V]
^{13}C-TCDF	Qual	315.94	251.97	26
^{13}C-TCDF	Quant	317.94	253.97	26
TCDF	Qual	303.89	240.94	26
TCDF	Quant	305.89	242.94	26
^{13}C-TCDD	Qual	331.94	267.97	20
^{13}C-TCDD	Quant	333.93	269.97	20
TCDD	Qual	319.89	256.93	20
TCDD	Quant	321.89	258.93	20
^{13}C-PeCDF	Qual	351.89	287.93	26
^{13}C-PeCDF	Quant	353.89	289.93	26
PeCDF	Qual	339.86	276.89	26
PeCDF	Quant	341.86	278.89	26
^{13}C-PCDD	Qual	367.89	303.93	22
^{13}C-PCDD	Quant	369.89	305.89	22
PeCDD	Qual	355.85	292.89	20
PeCDD	Quant	357.85	294.89	20
^{13}C-HxCDF	Qual	383.86	319.89	26
^{13}C-HxCDF	Quant	385.86	321.89	26
HxCDF	Qual	371.82	308.86	28
HxCDF	Quant	373.82	310.86	28
^{13}C-HxCDD	Qual	399.86	335.89	20
^{13}C-HxCDD	Quant	401.86	337.89	20

Table 1. Mass spectrometry parameters for the detection and confirmation of the analytes.

in the Extraluent cartridge and then into the silica SPE cartridge. The sample was transferred to a HRGC/HRMS 7200B Quadrupole Time-of-Flight GC/MS system (Agilent Thecnologies) with autosampler vial.

RESULTS AND DISCUSSION

In this study small-sized farms were monitored, from 10 to 100 heads. From the results obtained we can observe that on all the samples analyzed only three samples exceed the limit imposed by Regulation EC 1881/2006 which is equal to 3.0 pg/g fat. From the results obtained we can observe that on all samples analyzed only three samples exceed the limit imposed by Regulation EC 1881/2006 which is equal to 3.0 pg/g fat per sum of dioxins. The highest concentration value was 8.47 pg OMS-PCDD/F-TEQ/g fat, 3.75 pg OMS-PCDD/F-TEQ/g fat and 6.73 pg OMS-PCDD/F- TEQ/g fat. The results obtained from the determination of the other samples show very low levels of contamination from 1.36 to 0.0054 μg OMS-PCDD/F-TEQ/g by demonstrating that the milk produced is a safe food for human consumption (see also Schuhmacher et al., 2002; Gullet & Touai, 2003; Croes et al., 2013).

CONCLUSIONS

Positive results were obtained from samples taken from farms that use milk as raw material to be transformed into dairy products and then mixed with other raw materials with a dilution process that makes the product unholy to human health. Samples that show a level of dioxins above the law limit all fall into a wooded area where frequent fires develop which may result in increased concentrations first in the forage then transferring to milk.

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Biodiversity Journal, 2019, 10 (4): 465–468 https://doi.org/10.31396/Biodiv.Jour.2019.10.4.465.468
MONOGRAPH

Detection of Anisakidae larvae in fish products commercialized in Sicily

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ABSTRACTS

In this work a total of 1331 fish samples belonging to 15 species (*Engraulis encrasicolus*, *Alaccia aurita*, *Loligo vulgaris*, *Trigla lyra*, *Conger conger*, *Merluccius merluccius*, *Zeus faber*, *Lophius piscatorius*, *Sardina pilchardus*, *Lepidopus caudatus*, *Scorpaena scrofa*, *Scomber scombrus*, *Trachurus trachurus*, *Todarodes sagittatus*, *Trachinus draco*) from Sicilian commercialized in Sicily (FAO 37, FAO 37.1.3, FAO 37.1.1, FAO 37.2.2, FAO 37.3, FAO27,FAO 41, FAO87), were examined for the detection of Anisakidae larvae and molecular identification. The fish samples were examined for the research of nematodes by visual inspection and digestion method according to the EC Regulation 2075/2005. Detected larvae were subjected to morphological identification through the optical microscopy. Subsequently, the DNA was extracted and the molecular identification of the larvae was conducted by RFLP-PCR of the nuclear ITS region (ITS-1, ITS-2 and 5.8 S subunit). The polymerase chain reactions targeting the cytochrome c oxidase subunit II (cox2) was performed and subjected to sequence reaction. A number of 370 larvae have been identified at species level, of which 330 belong to the *Anisakis pegreffii*, 23 to the *Anisakis simplex* sensu strictu, 6 to the recombinant genotype *Anisakis pegreffii/simplex* s. s., 1 to *Anisakis physeteris* and 10 to *Hysterothylacium fabri*. The data obtained provide interesting reflections on the presence of parasites belonging to the Anisakidae family in fishery products marketed in the Sicilian territory. These findings are an excellent tool for assessing and preventing possible risks due to the consumption of these products.

KEY WORDS *Anisakis*; nematodes; larvae; PCR.

Received 22.11.2019; accepted 10.12.2019; published online 20.12.2019
Proceedings of the 4th International Congress on Biodiversity "Man, Natural Habitats and Euro-Mediterranean Biodiversity", November 17th-19th, 2017 - Malta

INTRODUCTION

In 2002, the Parliament of the European Union have developed a revision on the Community legislation about the production, marketing and food control, aimed to increasing the level of safety of food products. The EC Regulation 2002/178 laying down the general principles and requirements of food law and constituting the European Food Safety Authority (EFSA). Subsequently, a group of regulations, commonly called "hygiene package" were established from 1 January 2006. These regulations have redefined the existing EU provisions on hygiene of production and food marketing and have

set new rules on the food control for Competent Authorities and food sector operators. In recent years in Italy, we have seen an increasing consumption of raw fish due by importation of exotic typical products such as sashimi and sushi, and the increased consumption of different Italy culinary preparations such as marinated anchovies or carpaccio.

The Regulation 853/2004 imposes the food sector operators to freeze fish products eaten raw, this procedure ensuring food security. In fact there is a risk of parasites presence in fish fillets that can lead to zoonotical pathologies if larvae are eaten alive. The BIOHAZ panel of the European Food Safety Authority (EFSA recommended member states to carry out coordinated studies aimed to increase knowledge about the zoonotic diseases linked to Anisakis (Scientific Opinion on risk assessment of parasites in fishery products and EFSA Panel on Biological Hazards (BIOHAZ)). The study aims to estimate the prevalence of infestation of Anisakidae nematodes in the fish products commercialized and bred in Sicily and estimate the geographical and seasonal distribution of Anisakidae infestation

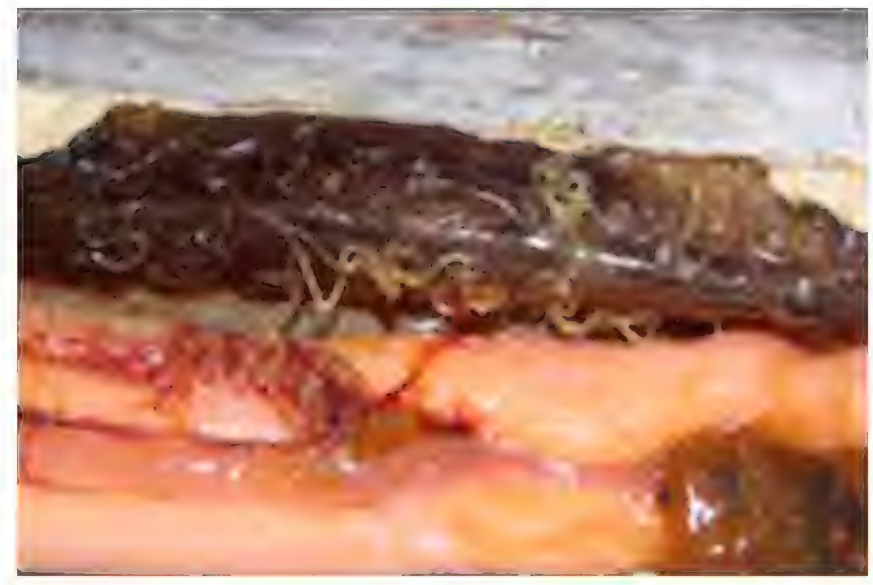

Figure 1. Anisakidae larvae infestation.

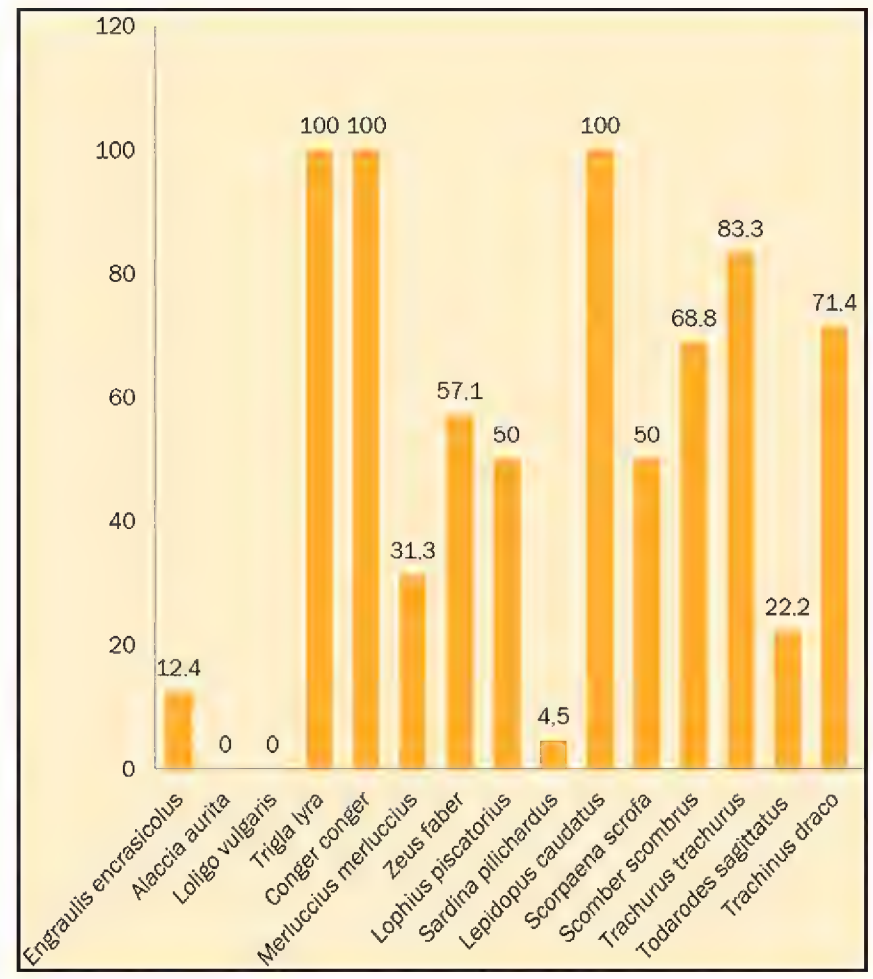

Figure 2. Infestation prevalence in the examined species.

MATERIAL AND METHODS

The fish samples were examined for the research of nematodes in the stomach, intestine, abdominal cavity and muscle by visual inspection and digestion method. Detected larvae are subjected to identification at genus level, through the optical microscopy (Leica DM 2000), according to morphological characters (Berland, 1961). Subsequently, the larvae were subjected to molecular analysis. Genomic DNA extraction were conducted by special kits based on the use of affinity columns, according to the manufacturer's instructions. Polymerase chain reactions targeting the complete ITS region ITS-1, 5.8S, ITS-2 and the cytochrome c oxidase subunit II (cox2) were performed. Purification of ITS and cox 2 gene amplification products was carried out with Illustra GFX PCR DNA and Gel Band Purification kit following the manufacturer's instructions. The purified products were sent to Macrogen company (Amsterdam, Holland) for Sanger sequencing. The sequences were compared with previously characterised ITS and cox2 sequences of Raphidascarididae family published for identification by using the Basic Local Alignment

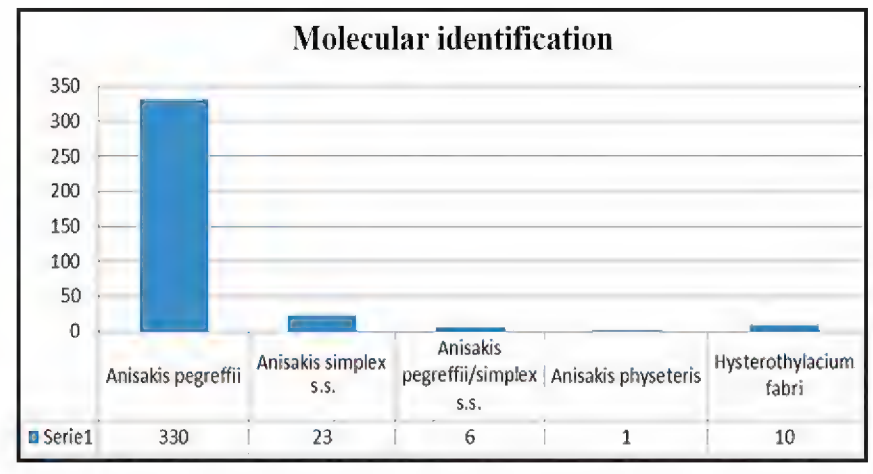

Figure 3. Molecular identificaton of Anisakidae larvae.

Species	FAO 37	FAO 37.1.3	FAO 37.1.1	FAO 37.2.2	FAO 27	FAO 37.3	FAO 41	FAO 87	Total
Alaccia aurita	31								31
Engraulis encrasicolus	434	114	85		75			88	796
Loligo vulgaris				10					10
Trigla lyra				5					5
Conger conger				8					8
Merluccius merluccius	49	3	13	15					80
Zeus faber				7					7
Lophius piscatorius	2			4					6
Sardina pilichardus	106		111	32	15				264
Lepidopus caudatus	3		2	3					8
Scorpaena scrofa	4			3		1			8
Scomber scombrus	11		10	2	9				32
Trachurus trachurus	11		26	5					42
Todarodes sagittatus	11	4		9			3		27
Trachinus draco	4			2		1			7
Totale				**1331**					

Table 1. Type and number of samples analysed.

Search Tool (BLAST) via GenBank™. Sequence Alignment was conducted using ClustalW 2.1 and MEGA 6.05 software. Evolutionary relationship among the *Hysterothylacium* haplotypes was analysed using Neighbor Joining (NJ) trees (Tamura et al., 2007). The evolutionary distances for NJ tree were computed using the Maximum Composite Likelihood method (Tamura et al., 2004).

RESULTS AND DISCUSSION

One thousand three hundred and thirty-one fish samples were examined for Anisakidae larvae detection by visual inspection (Table 1) The prevalence of infestation for each species examined were calculated (Fig. 1, 2). The collected larvae were examined for genre identification by optical microscopy, while the molecular identification was performed by RFLP-PCR (Fig. 3). The results obtained revealed the presence of Anisakidae larve in 450 fish samples. The comparison of the results obtained with Chaligianis have showed the lower values in the samples of *Merluccius merluccius* and *Scomber scombrus*, indicating a higher rate of infestation in fish species from the Greek coast. Molecular analysis confirm *Anisakis pegreffii* as the Anisakidae parasite most present in Mediterranean Sea (FAO zone 37-37.1-37.2-37.3) and also found in samples of *Engraulis encrasicolus* of from the North East Atlantic (FAO area 27) and in a sample of *Todarodes sagittatus* from the South Atlantic (FAO zone 41). Furthermore, the presence of *Anisakis simplex sensu strictu* has generally been identified in mackerel or in European anchovy from North-East Atlantic (FAO area 27) and only in one case in salted anchovies samples derived from the Mediterranean (FAO zone 37). The hybrid genotype (*Anisakis pegreffii*/*Anisakis simplex* s.s.) was found in samples from probable areas of sympathy (FAO 37.1.3; 37.1.1; 37.2) between the

Anisakis pegreffii and the *Anisakis simplex* s. s., in samples of *Trachurus trachurus*, *Scomber scombrus*, *Merluccius merluccius* and *Engraulis encrasicolus*. The species *Anisakis physeteris* was found in a single sample of *Conger conger* caught in FAO 37.2. The *Hysterothylacium fabri* has been identified in demersal fish samples such as *Conger conger*, *Lophius piscatorius*, *Trigla lyra*, *Trachinus draco*, *Merluccius merluccius* from FAO 37.2 and FAO 37.1 and in one case in one mackerel from the FAO area 27.

The results provide an exhaustive evaluation on the presence of Anisakidae parasites in fish caught and commercialized in Sicily in order to have a risk assessment associated with the consumption of these products (see also D'Amelio et al., 2000; 2010; Evans et al., 2001; Abollo et al., 2003; Bernardi, 2009; EFSA, 2010; Buchmann & Kania, 2012; Chaligiannis et al., 2012).

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Biodiversity Journal, 2019, 10 (4): 469–470 https://doi.org/10.31396/Biodiv.Jour.2019.10.4.469.470
MONOGRAPH

Sulphite's determination of Mediterranean Red Shrimp (*Aristaeomorpha foliacea*), in ionic chromatografy

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ABSTRACTS In the red shrimp (*Aristaeomorpha foliacea*), sulphites are added because they block the activity of an enzymatic complex responsible of shrimp's browing and maintain the aesthetic and commercial characteristics. An ionic chromatography after rapid distillation method was carried out for the quantitative determination of sulfites in food products. On 100 samples, only 0.7% were below the detection limit, 12.7% were above the limit allowed by law, 86.6% of samples were below the maximum permitted by law.

KEY WORDS Red shrimp; sulphites; chromatography.

Received 22.11.2019; accepted 10.12.2019; published online 20.12.2019
Proceedings of the 4th International Congress on Biodiversity "Man, Natural Habitats and Euro-Mediterranean Biodiversity", November 17th-19th, 2017 - Malta

INTRODUCTION

Sulphites are antimicrobial and antioxidant substances recognized as additives by the Commission Regulation 1129/2011.

In the red shrimp, *Aristaeomorpha foliacea* (Fig. 1), sulphites are added because they block the activity of an enzymatic complex called polyphenoloxidase (present in the shrimp cuticle) responsible of shrimp's browing.

These compounds are added to different food types in the form of Sulphur dioxide (E220), potassium metabisulphite (E224), sodium bisulphite (E222), potassium bisulphite (E228). The European Parliament and Council Directive 94/34/EC and subsequent amendments, regulates food additives permitted in the preparation and preservation of food.

They are used especially for the improvement of food well-appearance to make it more inviting for buyers because make it look fresher than it is.

In addition to these "free" sulphite species, formed in aqueous solutions, "bound" sulphites are also formed in foods by the reaction of sulfites with carbohydrate, protein and lipid molecules. These chemicals are harmful compounds which have mutagenic, cytotoxic and allergological effects.

MATERIAL AND METHODS

An ionic chromatography after rapid distillation method was carried out for the quantitative determination of sulfites in food products (Fig. 2).

The distillation was performed using vapor stream following acidification with HCl and addition of H_2O_2.

The distilled product was purified and injected into an ion chromatograph with Na_2CO_3 as eluent for the sulfites determination (mg SO_2 kg-1).

The linearity ranged from 4.4 to 400 mg SO_2 kg-1 with a correlation coefficient r2 = 0.9999.

The LOD and LOQ were 4.2 and 4.4, respectively. All the parameters of validation were in accordance with the EC Regulation on the analytical parameters used for the method reliability.

The method was accredited by the Italian national accreditation body and turned out to be much faster and more accurate than the conventional procedures.

Figure 1. Red shrimp, *Aristaeomorpha foliacea*.

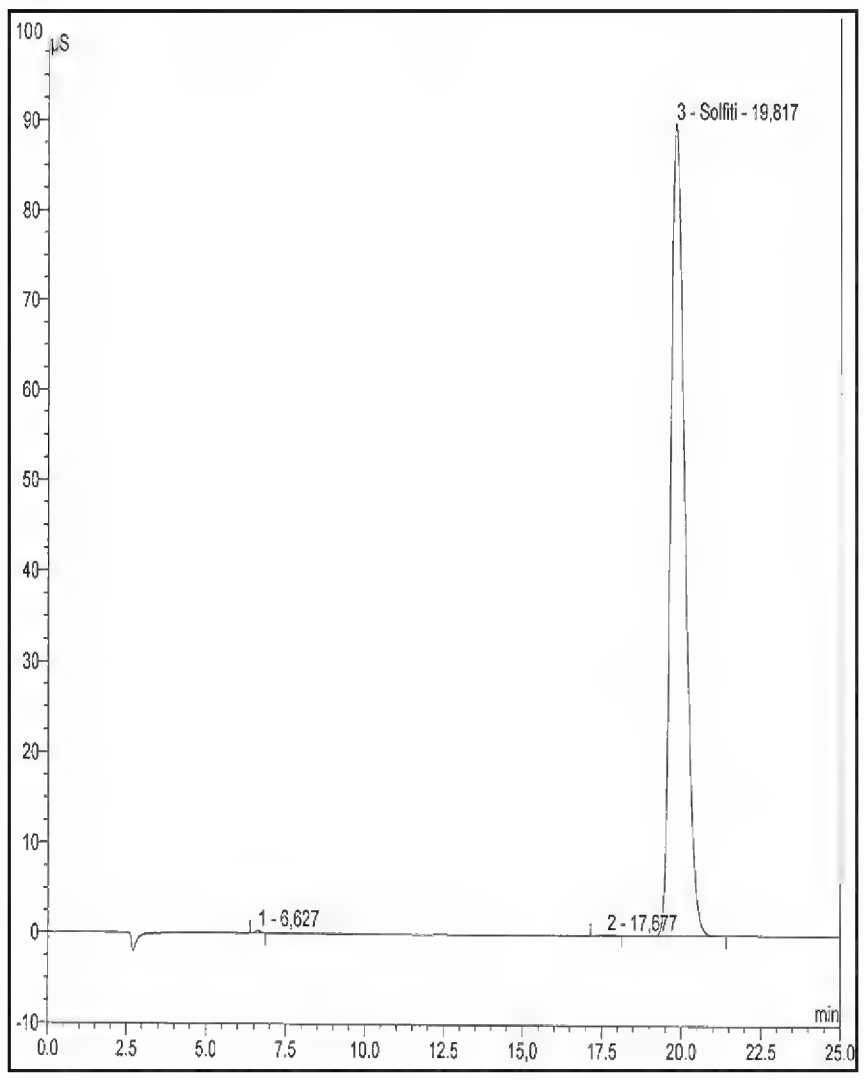

Figure 2. Chromatogram.

RESULTS

On a hundred specimens of the species in question, we revealed that only 0.7% are below the detection limit, 12.7% of the analyzed samples are above the limit allowed by law, in the remainder, quantized samples are below the maximum permitted by law.

DISCUSSION

The results obtained from the analyzes carried out on the red shrimp in question, it is clear that sulphites are used ubiquitously for the purpose of obtaining and maintaining the aesthetic and commercial characteristics.

To protect consumers (especially if they are intolerant to this chemical species), the law requires that the presence of this additive should be declared on the label. In addition, the method used for the analyzes in question was validated by an in-house validation model, according to the UNI CEI ENV 13005: 2000, UNI CEI EN ISO/IEC 17025:2005.

The method has a high recovery (> 98%) and does not interfere with the presence of interfering substances. See also cited references (Sullivan & Smith, 1985; Anderson et al., 1986; Campanella et al., 1990; Pizzoferrato et al., 1990).

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Biodiversity Journal, 2019, 10 (4): 471–473 https://doi.org/10.31396/Biodiv.Jour.2019.10.4.471.473
MONOGRAPH

Use of terrestrial gastropods (*Cornu aspersum*) as bioindicators of the environmental contamination status of the Sicilian Natural Parks to assess the contamination status of the pastures

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ABSTRACTS

The use of land molluscs in biomonitoring can evidence interactions between the contaminant and the biotic sphere. The concentration of heavy metals were determined by ICP-MS. In areas where anthropic and industrial contamination persist, heavy metal levels were above the quantification limit; sampling in agricultural areas shows lower levels of heavy metals. The same trend of results can be found for the analysis of IPAs. Snails can be considered as bioindicators of pollutants living in close contact with the soil and ingesting plants with a high concentration of contaminants.

KEY WORDS Snails; ICP-MS; heavy metals; IPA.

Received 22.11.2019; accepted 10.12.2019; published online 20.12.2019
Proceedings of the 4th International Congress on Biodiversity "Man, Natural Habitats and Euro-Mediterranean Biodiversity", November 17th-19th, 2017 - Malta

INTRODUCTION

Recently, with the transposition of Directive 2008/50 / EC (CAFE), attention has been paid to the use of bioindicators to assess changes in the quality of the environment. The organisms used as biomonitoring instruments should have specific sensitivity to certain environmental disturbances and are defined in the "biosensor" complex. Using bioindicator organisms it is possible to carry out an early environmental stress assessment and anticipate any interventions before major scale problems arise. As a result, they are an excellent monitoring tool. Some species of mollusks accumulate considerable amounts of metals and reflect bioavailable levels of the environment. For example, bivalve molluscs have long been used as bioindicators of contamination in aquatic environments rather than other animal groups. The use of land molluscs in biomonitoring also allows for evidence of interactions between the contaminant and the biotic sphere, which can not be determined solely by soil chemistry and ambient air analysis, which only identifies the quantity of pollutant that may be present, providing accurate data on contamination. Spikes can come into contact with environmental pollutants following skin contact with the soil, ingestion of soil, vegetation, water, air inhalation. They are sufficiently fixed to provide information related to the contamination of a site; in case of

bioaccumulation or physiological alterations, they would be used to evaluate in an integrated way the soil and air quality in terrestrial ecosystems. There are so many factors that affect the accumulation of metals, such as body weight, temperature, season and plants that feed. Many authors claim that molluscs of the same weight or size may be used for monitoring purposes and each metal has its own kinetics of intake and accumulation. Accumulation in the foot or the throat, the rate of growth and the mortality rate allow to define the environmental impact of contaminated soil on gastropods. To interpret the data of metal concentrations in gastropods, there is a classification of the concentrations of the metal found in the digestive gland based on the sampling environment. This classification, created by Pihan and also used by other authors, groups into concentrations in the digestive gland of the snails in the class and allows the site to be identified with greater contamination. This classification allows you to compare the results of your work with other existing ones in the literature and to understand the degree of contamination of the site being studied. In particular, the level of contamination of heavy metals (Cd, Pb, Cu, Zn, Cr) and polycyclic aromatic hydrocarbons (IPA) will be assessed in two Sicilian Natural Parks and the Park of the Sicani Mountains of New Institution through the use of terrestrial gastropods as environmental bioindicators.

MATERIAL AND METHODS

The concentrations of heavy metals were determined by an ICP-MS (7700x series, Agilent Technologies, Santa Monica CA, USA) equipped with octopole reaction system (ORS3). The sample solutions were pumped by a peristaltic pump from tubes arranged on an autosampler ASX-500 Series (Agilent Technologies, Santa Monica (CA), USA) and then conducted on a quartz cyclonic spray chamber. A fast and efficient sample digestion was achieved by a microwave-assisted system Multiwave 3000 (Anton-Paar, Graz, Austria) equipped with a rotor for eight MF100 PTFE-TFM (poly-tetrafluoroethylene-tetrafluoroethylene) vessels. The determination of IPAs was conducted in GC-MS/MS (Trace 1310 coupled with TSQ Quantum XLS Thermo Fisher Scientific) with the PTV

Figure 1. *Cornu aspersum*.
Figure 2, 3. ICP-MS Agilent series 7000.

split/splitless injector. The GC-MS/MS analysis for the determination of PAH were carried out according to the protocol of Sapozhnikova et al. (2013).

The choice of using this analytical technique has been determined for the low detection limits that have been obtained.

RESULTS AND DISCUSSION

The data obtained from heavy metal analyzes show low levels of contamination in relation to the sampling area (see also Cœurdassier et al., 2002 and Amaral et al., 2004).

Areas where anthropic and industrial contamination persist, heavy metal levels above the quantification limit (LoQ) have been found; sampling in agricultural areas shows lower levels of heavy metals than LoQ. The same trend of results can be found for the analysis of IPAs. In fact, sampling in high-density areas and in industrial areas has yielded quantifiable results, unlike the results obtained by sampling in purely agricultural and mountainous areas.

CONCLUSIONS

The results obtained show that there is a transfer

from the soil to the terrestrial gastropods of contaminants found. For this reason, snails can be used as bioindicators of the anthropic and industrial contamination levels of the territory where they were sampled. Furthermore, snails can be considered as carriers of pollutants living in close contact with the soil and ingesting plants with a high concentration of contaminants. Most contaminants are taken by gastroenteritis by ingestion. In particular, they focus on the digestive gland, which is the most important organ for both accumulation and detoxification. This organ is responsible for the production of digestive enzymes, absorption, digestion and endocytosis of food particles, as well as the storage and excretion of waste materials.

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Biodiversity Journal, 2019, 10 (4): 475–478 https://doi.org/10.31396/Biodiv.Jour.2019.10.4.475.478
MONOGRAPH

New report of *Anikasis* larvae from Blunthead Puffer, *Sphoeroides pachygaster* caught off Strait of Sicily

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ABSTRACTS

Aim of the present paper was to report and identify by morphological and molecular methods the presence of anisakid L3 larvae found in 7 specimens of *Sphoeroides pachygaster* caught off Strait of Sicily from 2012 and 2015. Nematode larvae (n=9) were collected from three fish samples: the larvae were morphologically identified as belonging to the genus *Anisakis* Type I and, stored in 70% ethanol, were underwent molecular identification at species level by PCR- RFLP analysis of the rDNA (ITS-1, 5.8S gene, and ITS-2) region. Sequencing of ITS regions and comparison with sequences in GenBank were also performed. The parasites were molecular identified as belonging to *A. pegreffii* that is the predominant species in Mediterranean Sea. This is a new report of *Anisakis* sp. in *S. pachygaster*. The blunthead puffer *Sphoeroides pachygaster* is a fish species of Atlantic origin: in the last years there are indeed numerous reports of this alien fish species in the Mediterranean included Italian Seas. The presence of the species *Anisakis pegreffii* may support the hypothesis of complete adaptation of *S. pachygaster* in the Mediterranean Sea.

KEY WORDS *Anisakis*; PCR-RFLP; nematodes.

Received 22.11.2019; accepted 10.12.2019; published online 20.12.2019
Proceedings of the 4th International Congress on Biodiversity "Man, Natural Habitats and Euro-Mediterranean Biodiversity", November 17th-19th, 2017 - Malta

INTRODUCTION

The blunthead puffer *Sphoeroides pachygaster* (Müller & Troschel, 1848) is widely distributed in tropical and temperate waters on both sides of the Atlantic Ocean (Shipp, 1990). The species is known in the Mediterranean Sea where in the last years several records were reported (Cherif et al., 2010; Hemida et al., 2009; Relini et al., 1995) included Italian Seas (Adriatic Sea, Tyrrhenian Sea, Ionian Sea, Sicilian Channel) (Vacchi & Cau, 1986; Arculeo et al., 1994; Giordano et al., 2012; Ragonese et al., 1997; Tursi et al., 1992; Ligas et al., 2006; Visentin & Borg, 2014). The presence of this alien species in the Mediterranean has been ascribed to the migration or casual transport through the Strait of Gibraltar and the Suez Canal; the spread is caused and favored by the ongoing phenomen of global warming (Massuti et al., 2010). Actually it is believed that *S. pachygaster* form a well established population in the Mediterranean Sea, with local reproduction, because of the discovery of young and adult specimens (Lipej et al., 2013; Ragonese et al., 2001).

There are not reported in the literature reports of parasitic nematodes of the Anisakidae family in blunthead puffer. This paper reports a parasitological survey of n. 7 specimens of this fish species, caught off Strait of Sicily, indicating at the same time for the first time the presence of nematode larvae of *Anisakis* sp.

MATERIAL AND METHODS

Fish samples were collected in the Strait of Sicily, in front of the Agrigento coast, during trawl surveys, from 2012 and 2015: 7 specimens of *S. pachygaster* were caught accidentally by fishing vessels. The specimens were transported refrigerated at the C.I.S.S. of the University of Messina and were identified, measured and weighted (Fig. 1).

On the studied fish, a parasitological exam was performed examining the coelomic cavity for metazoan parasites, by visual and stereoscopic inspection. Nematode larvae were collected, washed in saline solution, fixed in 70% ethanol and cleared with glycerol for morphological identification by light microscopy (Berland, 1961) (Fig. 3). The larvae, identified according to morphological characters as belonging to the genus *Anisakis*, were stored in 70% ethanol for underwent molecular identification at species level by PCR- RFLP analysis of the rDNA (ITS-1, 5.8S gene, and ITS-2) region using two restriction enzymes, HinfI and HhaI, for the identification of *Anisakis* spp. (D'Amelio et al., 2000; Pontes et al., 2005) (Fig. 4). Purification of ITS gene amplification products was carried out with Illustra GFX PCR DNA and Gel Band Purification kit (GE Healthcare) following the manufacturer's instructions. The purified products were sent to Macrogen company (Amsterdam, Holland) for Sanger sequencing. The morphological and molecular identification of the parasites were performed by the C.Re.N.A. of IZS of Palermo (Italy).

Figure 1. *Sphoeroides pachygaster*.

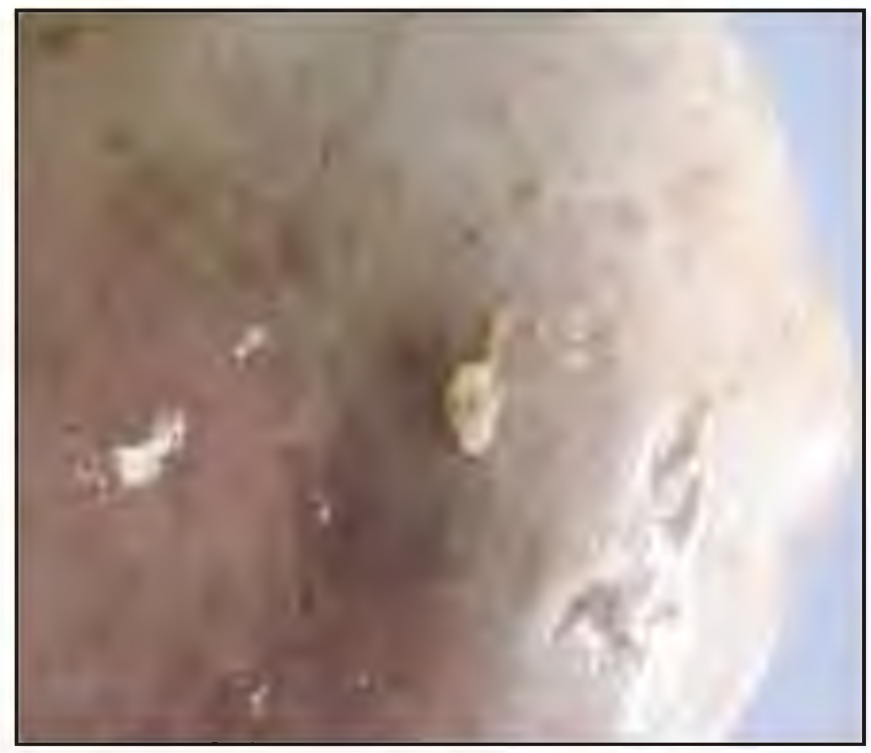

Figure 2. One larva encapsulated on the liver.

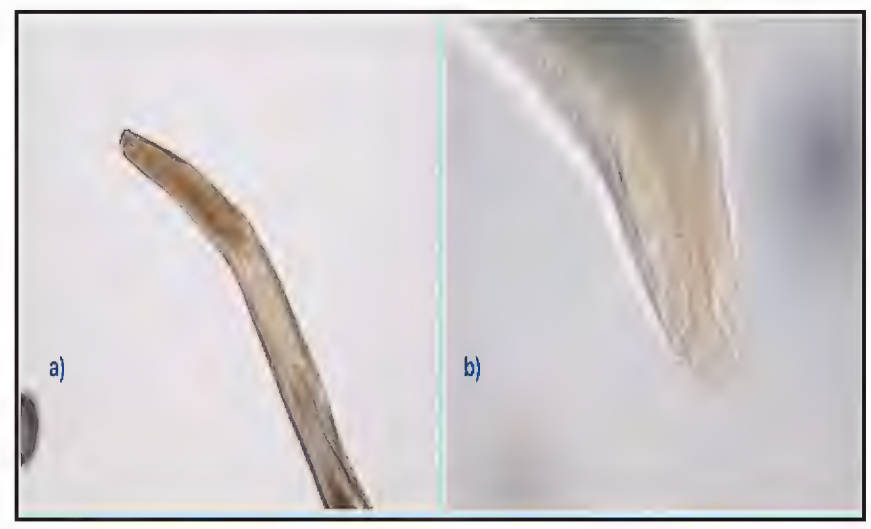

Figure 3. *Anisakis* larva Type I: a) anterior extremity b) tail showing mucron

RESULTS AND DISCUSSION

The results are reported in Table 1. Nematoda larvae (n= 9) were isolated in the coelomic cavity of 3 samples of *S. pachygaster*. Analysis of the morphological characteristics, observed by light microscopy, allowed to identify the parasites as *Anisakis* larvae Type I (sensu Berland, 1961) (Fig. 3). In the molec-

year	Samples n.	Weight g	Locality	Presence of parasites	Larvae isolated	Morphological identification	Molecular identification (PCR-RFLP)
2012	1	1750	Strait of Sicily	1	2	*Anisakis* Tipo I	*A. pegreffii*
2015	2	560	Strait of Sicily	1	3	*Anisakis* Tipo I	*A. pegreffii*
2015	3	940	Strait of Sicily	1	4	*Anisakis* Tipo I	*A. pegreffii*
2015	4	730	Strait of Sicily	-	-	-	-
2015	5	420	Strait of Sicily	-	-	-	-
2015	6	385	Strait of Sicily	-	-	-	-
2015	7	130	Strait of Sicily	-	-	-	-
Totale	**7**			**3**	**9**		

Table 1. Results of fish examined, year, weight and fishing locality.

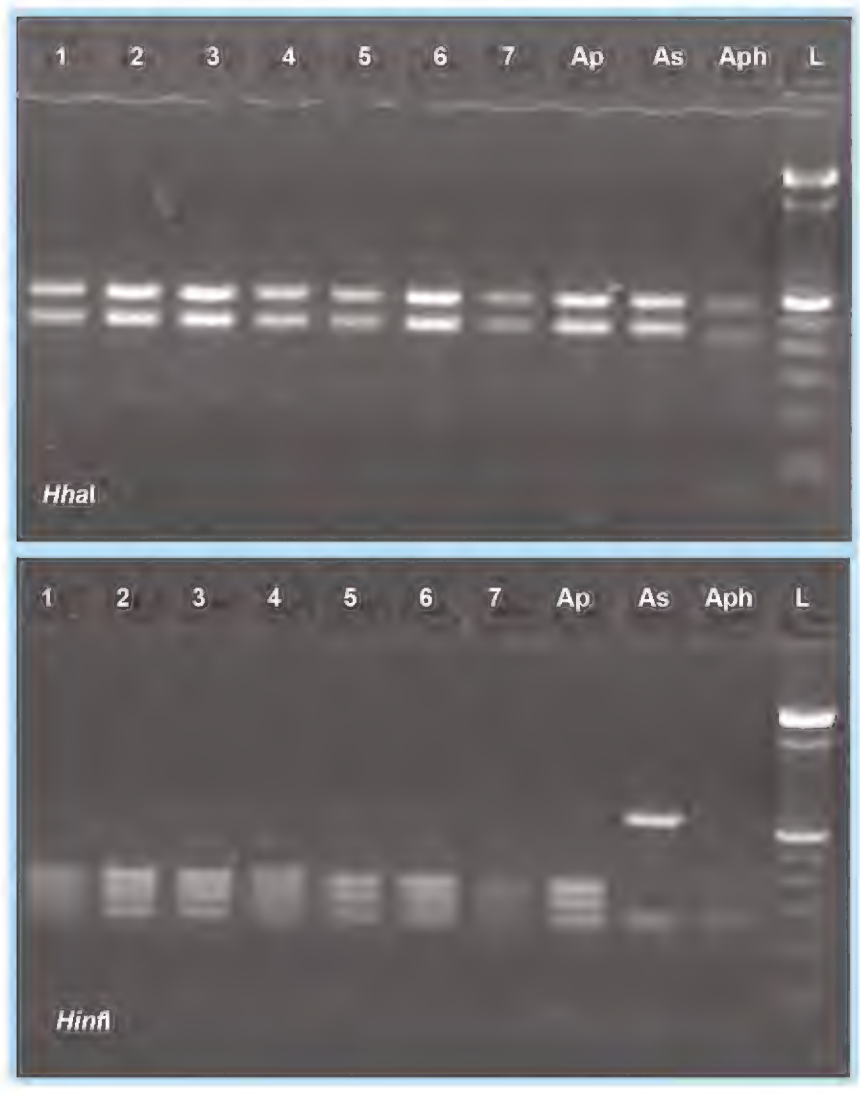

Figure 4. RFLP patterns obtained by digestion of the ITS region with the restriction enzymes Hha I and Hinf I. Lanes 1 7: *Anisakis pegreffii* (17), positive controls: Ap: *A. pegreffii*, *A. simplex* s.s., Aph: *A. physeteris* L: 100 bp ladder.

ular identification with PCR-RFLP analysis, the specific restriction profiles obtained were allowed to identify anisakid parasites as *Anisakis pegreffii*, according to the taxonomical keys available in literature (D'Amelio et al., 2000; Pontes et al., 2005). The sequences obtained were aligned and compared with the sequences deposited in GenBank, showing a 99% of identity with reference sequences of *A. pegreffii* (AY826720). *Anisakis pegreffii* is the predominant species in Mediterranean Sea while *A. simplex* s.s. is found in fish species of Atlantic Sea. The blunthead puffer *S. pachygaster* is a fish species of Atlantic origin, spread in all seas and oceans, warm and temperate. This alien species is known in the Mediterranean Sea where represent a definitively estabilished population. The discovery of anisakid nematodes and identification of species such as *A. pegreffii*, may support the hypothesis of complete adaptation of *S. pachygaster* as well as of existence of a Mediterranean population.

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Biodiversity Journal, 2019, 10 (4): 479–480 https://doi.org/10.31396/Biodiv.Jour.2019.10.4.479.480

MONOGRAPH

Presence of Ocratoxin (OTA) in wine produced in Sicily (Italy)

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ABSTRACTS Ocratoxin (OTA) occurs naturally in different foodstuffs, including grapes and their derivatives. OTA is a secondary fungal metabolite produced naturally by *Aspergillus* and *Penicillium* species. The purpose of this work was to estimate the occurrence of OTA in wine produced in Sicily to obtain a risk assessment of these substances. A total of 470 wine samples were quantitatively analysed by screening analysis method based on immunoenzymatic assay (ELISA) for ochratoxin. Results confirmed the presence of contamination by OTA in only one sample of red wine "Nero D'Avola" from south-oriental Sicily.

KEY WORDS Ochratoxins; wine; ELISA.

Received 22.11.2019; accepted 10.12.2019; published online 20.12.2019
Proceedings of the 4th International Congress on Biodiversity "Man, Natural Habitats and Euro-Mediterranean Biodiversity", November 17th-19th, 2017 - Malta

INTRODUCTION

Ocratoxin (OTA) occurs naturally in different foodstuffs, including grapes and their derivatives. The first reports of OTA in wine go back to 1996. Since then, many studies have focused on OTA occurrence in products derived from grapes such as dried vine fruit, wine, grape juice, must and vinegar. Red wines have been reported to be contaminated more frequently than white wines, probably due to the different wine-making methods involved. OTA occurrence seems to be higher in wines from Southern European countries: several studies have shown an increase in the amount of OTA in warmer climates. Given the danger posed by OTA, the European Community has recently established a concentration limit of 2 µg/kg in grape juice, must, wine and dried fruit (Commission Regulation n. 1881/2006/EC). OTA is a secondary fungal metabolite produced naturally by several *Aspergillus* and *Penicillium* species; *Aspergillus* and *Penicillum* species able to produce OTA occur in temperate and cold climate areas, respectively. The most important OTA-producing species belong to *Aspergillus* sections *Circumdati* and *Nigri*; however, the presence of OTA in grapes and wine is mainly linked to the contamination in the vineyard by species belonging to the *Aspergillus* section *Nigri*, the so-called black aspergilli. The major producer of OTA in grapes is *Aspergillus carbonarius*, though other species belonging to the Nigri and Circumdati sections have also been found to produce the toxin in different Mediterranean countries, such as Spain, Italy, Portugal, in Australia and South America (see also IARC, 1993; Majerus & Otteneder, 1996; Pfohl-Leszkowicz & Manderville, 2007).

The purpose of this work was to estimate the occurrence of OTA in wine commercialized and pro-

Figure 1. Sicilian wine.

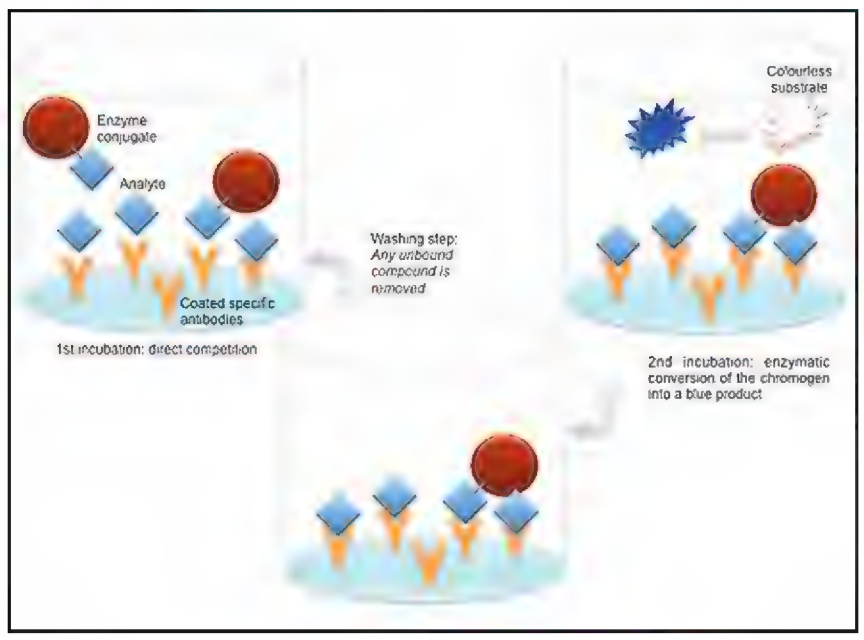

Figure 2. ELISA scheme functioning.

duced in Sicily to obtain a detailed risk assessment of these substances.

MATERIAL AND METHODS

A total of 470 wine samples based were analised by screening analysis method based on immunoenzymatic assay for the quantitative analysis of ochratoxin. The assay is performed in polystyrene microwells which have been coated with antibodies (IgG) anti -IgG of rabbit. Ocratoxin A standard solution or samples, the enzyme conjugate ochratoxin-HRP and a specific antibody anti-ocrhatoxin were added to the microwells. During the incubation, free ochratoxin-A mulecules and ochratoxin - HRP compete for the anti - ochratoxin antibodies binding sites. The anti-ochratoxin antibodies are simultaneously bound to the solid fase. Any unbound enzyme ochratoxin - HRP is then removed in a washing step.

The bound enzyme (HRP) activity is determined by adding a fixed amount of a chromogenic substrate. The enzyme converts the colourless chromogen into a blue product. The addition of the stop reagent leads to a color change from blue to yellow. The absorbance was measured by a microplate reader at 450 nm. The colour development is inversely proportional to the ochratoxin-A concentration in the sample (Figs. 1, 2).

RESULTS

The results of the present work confirmed the presence of contamination by OTA in only one sample of red wine "Nero D'Avola" from south-oriental Sicily. Specifically in the year 2017 they were examined at the laboratories of the IZS Sicilia in the area of Chemical and Food Technologies 29 samples of officially withdrawn ASP from Sicilian territory. Of these samples 28 are below the detection limit of the method, while only one sample was found to be inadequate to ELISA screening because it exceeded the legal limit established by the Reg. 1881/2006/CEE. The results confirmed good standard practices of Sicilian industry for wine production.

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Biodiversity Journal, 2019, 10 (4): 481–484 https://doi.org/10.31396/Biodiv.Jour.2019.10.4.481.484
MONOGRAPH

Evaluation of Histamine level in the Red Tuna (*Thunnus thynnus*) of the Mediterranean Sea in 2010–2015

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ABSTRACTS

Histamine is a biogenic amine present in fish species associated with a high amount of histidine, and it can cause the 'scombroid poisoning'. The Commission Regulation (EC) No 2073/2005 govern the criteria to analyze histamine in fishery products and specifies that the high performance liquid chromatography (HPLC) is the reference method. In this work, 664 samples were presented in the 2010–2015 shine, of which 46 (6.9%) were positive. With Chart 1 it can be noted that the number of samples has increased substantially in recent years (2013–2015) due to intensified official controls. On the contrary, the number of positives has decreased progressively.

KEY WORDS Histamine; red tuna; mediterranean; HPLC.

Received 22.11.2019; accepted 10.12.2019; published online 20.12.2019
Proceedings of the 4th International Congress on Biodiversity "Man, Natural Habitats and Euro-Mediterranean Biodiversity", November 17th-19th, 2017 - Malta

INTRODUCTION

Food safety is a very subject matter nowadays, in fact, the attention of consumers on the risks of ingestion of harmful compounds through food grows more and more every day. The fish is an essential food for a complete diet, but being particularly rich in protein and free amino acids, is more subject to decomposition processes that could lead to the formation of substances harmful to consumers. Among these, there is the Histamine, a biogenic amine, derived from the decarboxylation by fish proteolytic bacteria of the amino acid Histidine. Histamine causes the so-called scombroide syndrome (Hungerford, 2010). This syndrome is usually a mild illness characterized by rash, hives, nausea, vomiting, diarrhea, flushing, tingling and itching of the skin (Taylor, 1986), the severity of symptoms varies depending on the amount of histamine assumed and individual susceptibility. For these reasons the histamine levels should be monitored in particular in fish that contain high levels of histidine in their tissues, particularly fish species of the families: Scombridae, Clupeidae, Engraulidae, Coryfenidae, Pomatomidae, Scombresosidae, such as tuna, mackerel, anchovy and sardines. An inappropriate treatment of these fish, at temperatures above 4 °C, during the storage or processing stages does increase the histamine levels due to degradative processes. Once produced, histamine tends to remain unchanged in the food, since it is particularly resistant to heat and it is not destroyed by normal cooking temperatures.

The Commission Regulation (EC) No 2073/2005 of November 15th, 2005 on "Microbiological criteria for foodstuffs" and the subsequent Commission Regulation (EC) No 1441/2007, govern the criteria to analyze the Fishery products from fish species associated with a high amount of histidine. It specifies that the sampling plan includes nine aliquots and that the high performance liquid chromatography (HPLC) is the reference method that must be used to detect the histamine.

These regulation limit histamine content and evaluate the test results:

- satisfactory, if all the values observed are ≤ 100 mg/Kg,

- acceptable, if a maximum of 2 values are between 100 and 200 mg/Kg, and the rest of the values observed are ≤ 100 mg/Kg,

- unsatisfactory, if one or more of the values observed are > 200mg/Kg or more than 2 values are between 100 mg/Kg and 200 mg/Kg.

For products that have undergone enzymatic maturation in brine, fitness values are doubled. Analytical analysis is needed to evaluate the deterioration of fishery products, particularly fresh fish, where the possible use of new generation illicit additives such as Cafodos® that alters sensory evaluation (Muscarella et al., 2013; Piersanti et al., 2014). Histamine monitoring was accepted globally for the confirmation of the safety of fishery products (Tao et al., 2011). The purpose of this study was to evaluate the content of Istamine in the Red Tuna-Mediterranean Sea (*Thunnus thynnus*) of FAO 37.576 - 12.326 North latitude, received in the laboratories of the Department of Chemistry and Food Technologies of the Zooprophylactic Institute Experimental of Sicily in the years 2010–2015.

MATERIAL AND METHODS

A total of 664 Tuna samples from FAO 37.576-12.326 North latitude, were examined at the laboratories of the Department of Chemistry and Food Technologies of the "Istituto Zooprofilattico Sperimentale della Sicilia" (Italy) over the years 2015–2016.

The internal method was validated according to UNI CEI EN ISO / IEC 17025 standard by fortifying tuna samples on three levels of histamine (100, 200, 400 mg / kg) by performing ten replicas at each validation level to determine repeatability, uncertainty and robustness. Ten low fortified samples (10 mg/kg) were used for measuring the detection limit (LOD) and the quantification limit (LOQ). The linear response of the method was verified in histamine levels from 10 to 100 mg/L, with a determination coefficient (R2) equal to 0.9995.

Reagents and Equipment

Histamine dihydrochloride, sodium 1-decanesulfonate, potassium monophosphate, potassium hydrogenphosphate trihydrate, acetronitrile, perchloric acid were purchased from Sigma- Aldrich. All the chemical reagents and solvents were of analytical and chromatographic grade, respectively. Ultrapure water was obtained from a Millipore purification system.

A UHPLC Agilent 1290 series (Waldbronn, German) system and a chromocrophic column of Supelcosil LC-ABZ 15 cm, 4.6 mm. ID, 5 µm a was used for the analyzes

Sample preparation ad extraction

The samples were previously homogenized and weighed (10 g) in a 50 mL centrifuge test-tube. A sample was fortified by adding of 2 ml of the histamine standard solution at 1000 mg/L (final concentration 200 mg/kg). To the weighted sample was added 10 ml of 6% perchloric acid solution and the mixture was vortexed for 1 minute, then were added 30 mL of deionized water and the mixture was vortexed for 1 minute, centrifuged for 10 minutes at 3000 rpm and the supernatant was filtered on a 0.45 mm microfilter directly into vials.

High performance liquid chromatography conditions

The chromatographic separations were run with an UPLC Agilent 1290 with UV/DAD detector on a Supelcosil LC-ABZ column (15 cm, 4.6 mm. DI 5 mm) . Injection volume was 20 µL, the flow rate was 1.2 mL/min at room temperature and the detector wavelength was set to 210 nm. The method involved an isocratic elution using a Mobile phase A consisted of the Phosphate buffer solution at pH 6.9 and mobile phase B consisted of acetonitrile (85:15, v/v).

Solution	mg/L	Volume solution to be taken		Final volume	Solvent
Mother sol.	1000	167 mg	oltdo	100 mL	H_2O
SMix1	150	1500 µL	Mother sol.	10 mL	H_2O
SMix2	100	100 µL	Mother sol.	10 mL	H_2O
SMix3	50	500 µL	Mother sol.	10 mL	H_2O
SMix4	20	200 µL	Mother sol.	10 mL	H_2O
SMix5	10	1000 µL	SMix2	10 mL	H_2O

Table 1. Standard solutions.

Standard solutions

Standard solutions were prepared according to Table 1.

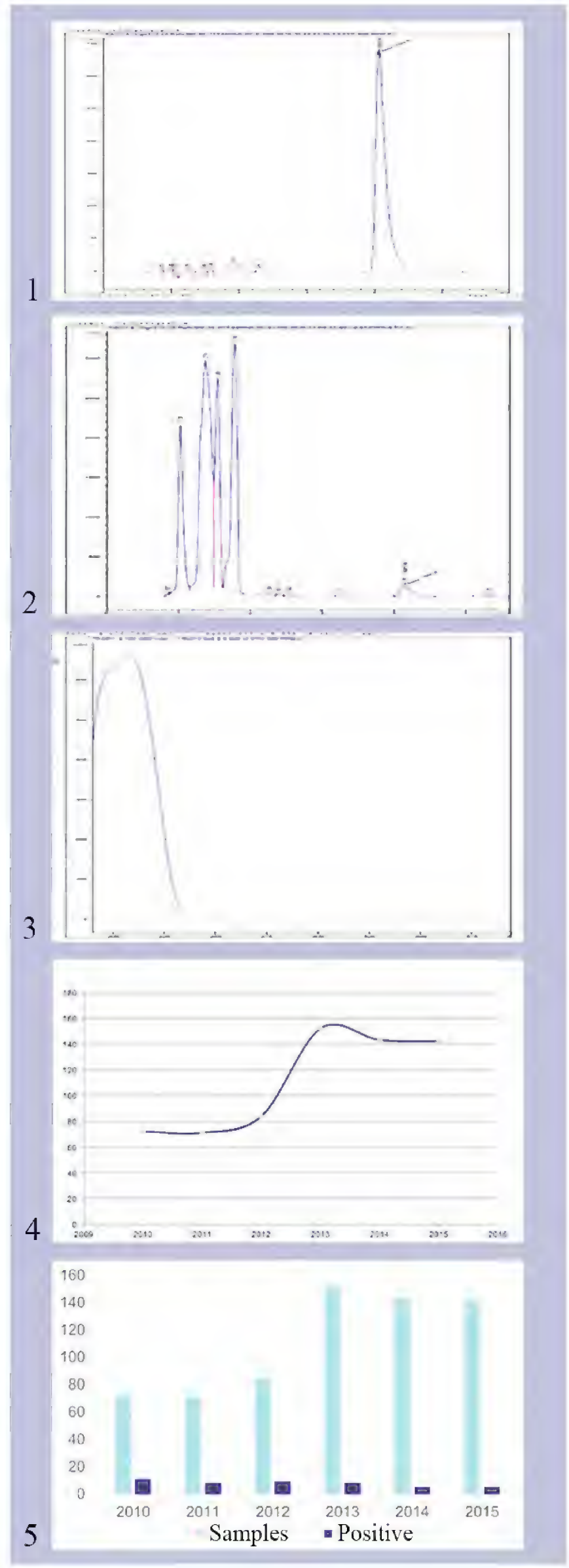
RESULTS

The HPLC-DAD chromatogram of a histamine standard solution 200 mg/L is shown in Figure 1; you can see a unique and recognizable peak with a 4 minute retention time relative to histamine. While Figure 2 shows a typical HPLC-DAD chromatogram on a fortified sample, it is possible to note that there are other spikes associated with the matrix and, at 4 minute time, the peak of the analyte in question. The presence of the analyte can be further verified by superimposing UV-vis spectra. In Figure 3, a typical UV-Vis spectrum of histamine is shown, showing absorption between 200 and 240 nm with a maximum of $\lambda = 210$ nm

CONCLUSIONS

In this work, 664 samples were presented in the 2010–2015 shine, of which 46 (6.9%) were positive. With Chart 1 it can be noted that the number of samples has increased substantially in recent years (2013–2015) due to intensified official controls. On the contrary, the number of positives has decreased progressively.

Samples from the Mediterranean are fairly controlled and free from histamine, while cases of scombroid syndrome that are occurring are probably due to the poor conservation of fish by end consumers (and end-users).



Figures 1–5. Explanation of the figures in the text (Results).

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Biodiversity Journal, 2019, 10 (4): 485–488 https://doi.org/10.31396/Biodiv.Jour.2019.10.4.485.488
MONOGRAPH

Molecular identification of larvae for Anisakidae family reduced in benthic and pelagic fish

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ABSTRACTS The present work, carried out at the Istituto Zooprofilattico Sperimentale della Sicilia "A.Mirri", Department of Food, Laboratory C.Re.NA (National Reference Center for Anisakiasis), aims to identify species of larvae found in different fish species from the pelagic and benthic environment through molecular analysis (PCR, PCR-RFLP and sequencing) and evaluate the possible ecological relationship between parasites and guests in the two different marine environments.

KEY WORDS Anisakidae; PCR-RFLP; benthic; pelagic.

Received 22.11.2019; accepted 10.12.2019; published online 20.12.2019
Proceedings of the 4th International Congress on Biodiversity "Man, Natural Habitats and Euro-Mediterranean Biodiversity", November 17th-19th, 2017 - Malta

INTRODUCTION

In the last ten years, there was an increase in the consumption of raw fish due to the introduction of new eating habits from countries outside Europe (sushi, sashimi, herring, pickled anchovies, etc.), increasing the risk of contracting parasitic diseases, such as Anisakiasis (Audicana et al., 2002). Anisakiasis is a disease caused by nematodes of the genus *Anisakis*, belonging to the Anisakidae family, together with *Pseudoterranova*, genera (Køie et al., 1995; Pozio, 2013). Human infestation occurs by ingestion of third stage *Anisakis* larvae present in raw fish and cephalopods but also undercooked, marinated, pickled, smoked or salted. These parasites are found, at the adult stage, in the abdomen of marine mammals (whales, seals, dolphins), more precisely in the stomach where they are visible to the naked eye. In fish, which are intermediate hosts, the larval form are normally found in the coelomic cavity where they can found free or encysted or adherent to the various organs and tissues (Mattiucci & Nascetti, 2008). In the present study larval forms of Anisakidae collected in pelagic, benthic and demersal fishes sampled in Sicily were identified by molecular analysis according to the protocols reported in literature (Cavallero et al., 2015).

MATERIAL AND METHODS

Detected larvae are subjected to identification at genus level, through the optical microscopy (Leica DM 2000), according to morphological characters (Berland, 1961). Subsequently, the larvae were washed, fragmented with a scalpel and frozen at -20 °C for 24 hours. The extraction of DNA were conducted by special kits based on the use of affin-

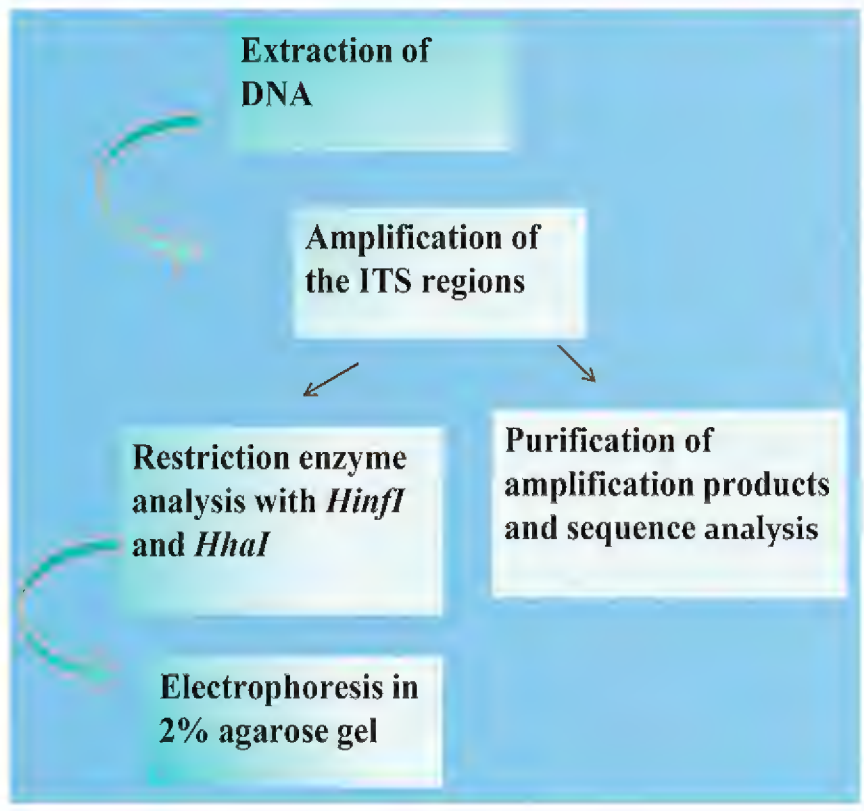

Figure 1. Molecular identification.

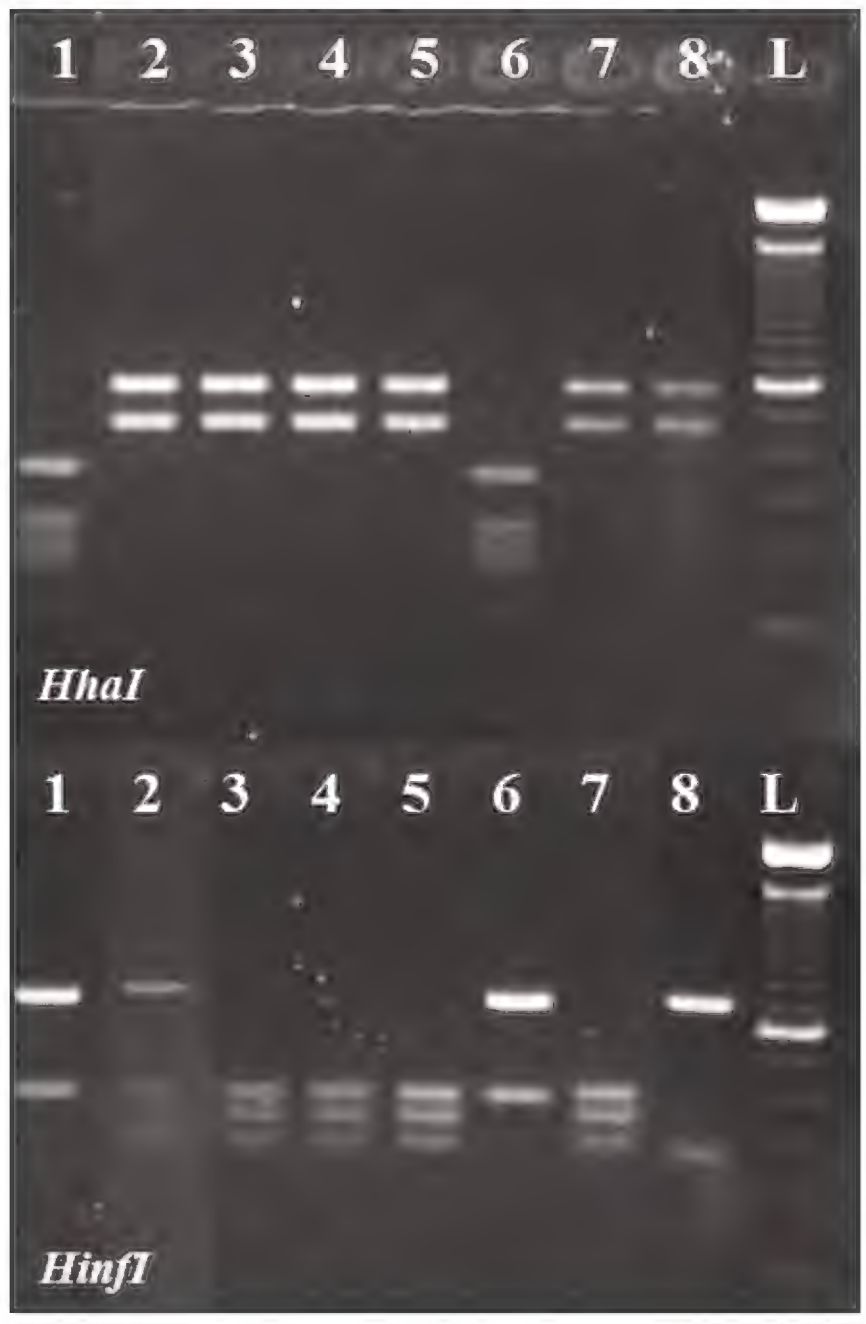

Figure 2. Restriction enzyme analysis with HinfI and HhaI Lane 1-6: *Anisakis* typica; lane 2: *Anisakis simplex* s.s./*Anisakis pegreffii* hybrid form; lane 3-4-5-7: *Anisakis pegreffii*; lane 6: *Anisakis simplex* s. s.; L: ladder 100 bp.

ity columns. For the genus *Anisakis* we proceeded to the amplification of the ITS regions (ITS-1, ITS-2 and 5.8 S subunit) of nuclear rDNA by the primers NC5 (5'GTA GGT GAA CCT GCG GAA GGA TCA TT3'), NC2 (5'TTA GTT TCT TTT CCT CCG CT3') (Zhu et al., 1998). The DNA samples have been subjected to restriction enzyme analysis with two restriction enzymes, HinfI and HhaI, for the identification of *Anisakis* spp. according to genetic key of D'Amelio et al. (2000). The digestion was performed over night at 37°C and the digestion products were electrophoresed in 2% agarose gel (Invitrogen) stained with SYBR safe® and visualized by UV transilluminator (Figs. 1, 2). For the identification of *Hysterothylacium* species a sequence analysis were conducted. Purification of ITS gene amplification products was carried out with Illustra GFX PCR DNA and Gel Band Purification kit (GE Healthcare) following the manufacturer's instructions. The purified products were sent to Macrogen company (Amsterdam, Holland) for Sanger sequencing (Fig. 3).

RESULTS AND DISCUSSION

In the seas surrounding Sicily the larvae found in the various species of fish belonged to the *Anisakis* and *Hysterotilacium* genera of the Fischer Anisakidae. The pelagic environment was rich in larvae belonging to the *Anisakis* genus such as *Anisakis pegreffii*, *Anisakis simplex* s.s. as well as the hybrid species *Anisakis pegreffii* / *Anisakis simplex* s.s. In the benthic environment predestined species belonging to the genus *Hysterotilacium* there was also a low presence of larvae of the species *Anisakis pegreffii*. Demersal fish species were found infested both by *Hysterotilacium aduncum* and by *Anisakis pegreffii*. The data obtained indicate a different parasites belonging to the Anisakidae family as well as the fish species of pelagic and benthic marine environments, indicating therefore a difference of infestation in relation to the different habitats (Fig. 4).

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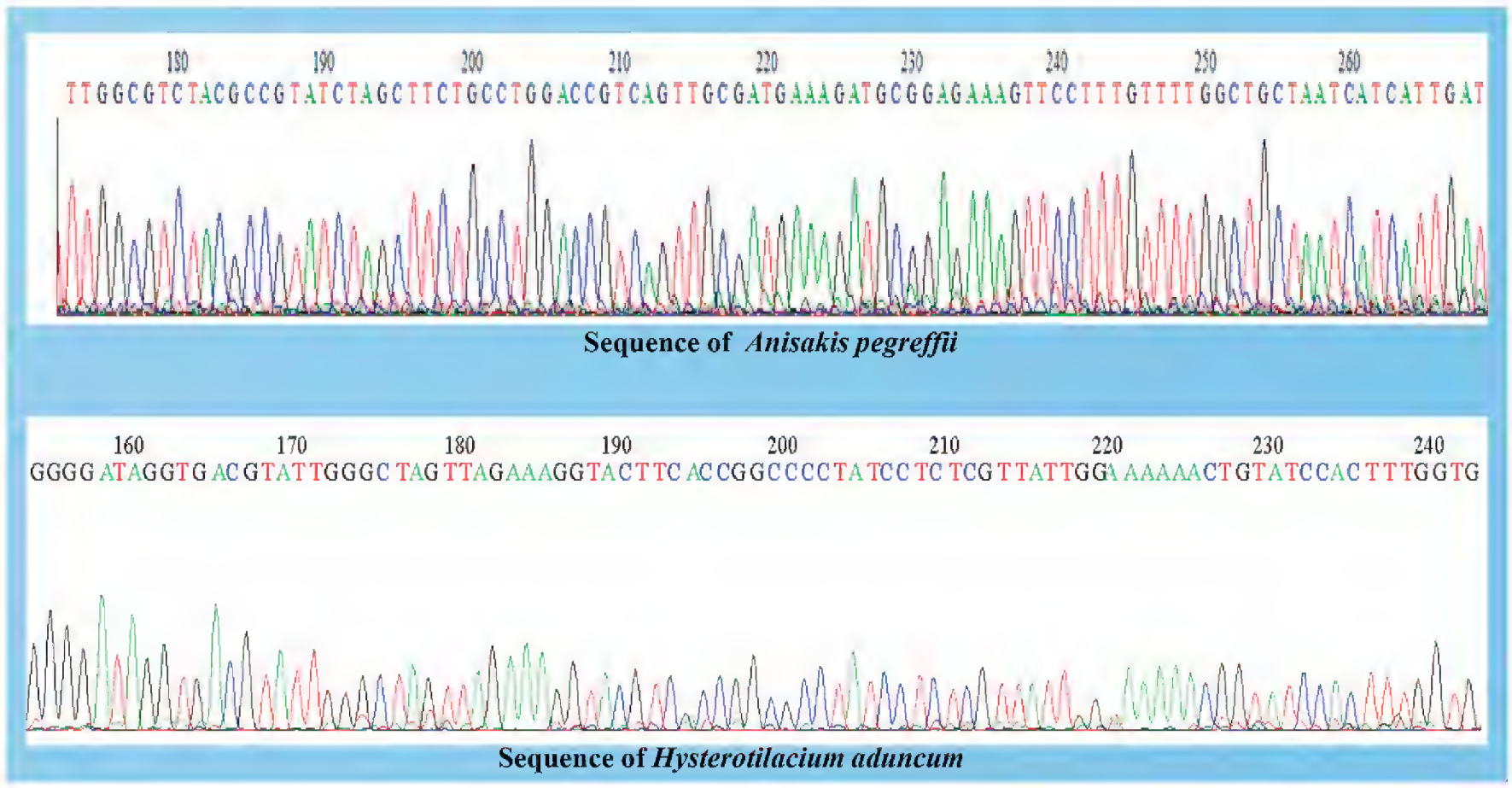

Figure 3. Sanger sequencing.

	Species	n. guests	n. larvae	Larvae species	zone F.A.O.	
Pelagic environment	*Sgomber sgombrus* *Engraulis encrasicolus* *Sardina pilchardus*	289	259	*A. pegreffii* *A. simplex* *A. pegreffii/A. simplex*	37.12 37.19 37.15 27	TP ME SR Atlantic
Benthic environment	*Mullus barbatus* *Scorpaena scrofa*	29	8	*H. aduncum* *A. pegreffii*	37.16 37.15	AG SR
Demersal	*Merlucius merlucius* *Lepidopus caudatus*	9	604	*A. pegreffii* *H. aduncum*	37.12	TP
Total		327	871			

Figure 4. Larvae of Anisakidae family found in bentonic and pelagic fish.

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Biodiversity Journal, 2019, 10 (4): 489–490 https://doi.org/10.31396/Biodiv.Jour.2019.10.4.489.490
MONOGRAPH

Sulphite's determination in equine meat and its preparations

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ABSTRACTS Sulphites are antimicrobial and antioxidant substances recognized as additives. Furthermore, these chemicals may reduce the nutritional quality of food by interacting with some vitamins such as nicotinamide, folic acid, thiamine and pyridoxal. An ionic chromatography with conductivity suppressor detector, after rapid distillation method was carried out for the quantitative determination of sulfites in food products. On two hundred samples of equine meat, we revealed that 23% of the analyzed samples are above the limit allowed by law. Sulphites are used illegally with the purpose of obtaining and maintaining the aesthetic and commercial characteristics.

KEY WORDS Sulphites; meat; chromatography.

Received 22.11.2019; accepted 10.12.2019; published online 20.12.2019
Proceedings of the 4th International Congress on Biodiversity "Man, Natural Habitats and Euro-Mediterranean Biodiversity", November 17th-19th, 2017 - Malta

INTRODUCTION

Sulphites are antimicrobial and antioxidant substances recognized as additives by the Commission Regulation 1129/2011.

These compounds are added to different food types in the form of Sulphur dioxide (E220), potassium metabisulphite (E224), sodium bisulphite (E222), potassium bisulphite (E228). The European Parliament and Council Directive 94/34/EC and subsequent amendments, regulates food additives permitted in the preparation and preservation of food.

They are used especially for the improvement of food well-appearance to make it more inviting for buyers because make it look fresher than it is.

In addition to these 'free' sulphite species, formed in aqueous solutions, 'bound' sulphites are also formed in foods by the reaction of sulfites with carbohydrate, protein and lipid molecules.

These chemicals are harmful compounds which have mutagenic, cytotoxic and allergological effects.

Sulphites were included in the allergens list by the Food and Drug Administration of the European Commission and must be mentioned on the food label if its concentrations are above 10 mg kg-1 (expressed as SO_2). Therefore, for prepacked foods, their presence in a food or beverage must be indicated on the label, by its full name, where the level exceeds 10 mg kg-1 or mg l-1 (expressed as SO_2).

Furthermore, these chemicals may reduce the nutritional quality of food by interacting with some

vitamins such as nicotinamide, folic acid, thiamine and pyridoxal.

A daily tolerable intake of sulphites was defined as 0.7 mg kg-1 body weight by the Food and Agriculture Organization.

MATERIAL AND METHODS

An ionic chromatography with conductivity suppressor detector, after rapid distillation method was carried out for the quantitative determination of sulfites in food products.

The distillation was performed using vapor stream following acidification with HCl and addition of H_2O_2.

The distilled product was purified and injected into an ion chromatograph with Na_2CO_3 as eluent for the sulfites determination (mg SO_2 kg-1).

The linearity ranged from 4.4 to 100 mg SO_2 kg-1 with a correlation coefficient r2 = 0.9999.

The LOD and LOQ were 4.2 and 4.4, respectively.

All the parameters of validation were in accordance with the EC Regulation on the analytical parameters used for the method reliability. The method was accredited by the Italian national accreditation body and turned out to be much faster and more accurate than the conventional procedures.

Figure 1. Equine meat.

RESULTS

Of the 200 equine meat samples analysed 46 showed sulphites presence with very high concentrations.

DISCUSSION

Was found by our analysis, that sulphites are used illegally, especially in equine meat preparations for the purpose of obtaining and maintaining the aesthetic and commercial characteristics; as they are most exposed to oxidation reactions. The method used for the analyzes in question was validated by an in-house validation model, according to the UNI CEI ENV 13005:2000 , UNI CEI EN ISO/IEC 17025:2005 (see also: Sullivan & Smith, 1985. Anderson et al., 1986; Pizzoferrato et al., 1990; Ravi et al., 1999; Iammarino et al., 2012).

The method has a high recovery (> 98%) and does not interfere with the presence of interfering substances.

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Biodiversity Journal, 2019, 10 (4): 491–492 https://doi.org/10.31396/Biodiv.Jour.2019.10.4.491.492
MONOGRAPH

Determination of Chlorpyriphos in Sicilian peaches by Gascromatography-MSMS method coupled with quechers sample preparation procedure preparation

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ABSTRACTS Chlorpyrifos is an organophosphate pesticide used to kill a number of pests including insects and worms. It is considered moderately hazardous to humans by the World Health Organization and is harmful for these classes of animals. Gas chromatography tandem mass mass spectrometry (GC-MS) was used for the quantification and confirmation of Chlorpyrifos pesticide residues in peaches. The analysis carried out on peach samples confirmed that chlorpyrifos are widely used in Sicilian territory so that 34 percent of the samples were contaminated by the pesticide.

KEY WORDS Chlorpyrifos; pesticides; GC-MS.

Received 22.11.2019; accepted 10.12.2019; published online 20.12.2019
Proceedings of the 4th International Congress on Biodiversity "Man, Natural Habitats and Euro-Mediterranean Biodiversity", November 17th-19th, 2017 - Malta

INTRODUCTION

Chlorpyrifos, sold under many brandnames, is an organophosphate pesticide used to kill a number of pests including insects and worms. It is used on crops, animals. It acts on the nervous system of insects by inhibiting acetylcholinesterase. Chlorpyrifos is considered moderately hazardous to humans by the World Health Organization. Exposure surpassing recommended levels has been linked to neurological effects, persistent developmental disorders and autoimmune disorders. Exposure during pregnancy may harm the mental development of children. In agriculture, it is one of the most widely used organophosphate insecticides in Sicily (Lehotay, 2010; Wilkowska & Biziuk, 2011).

The aim of this work is analyzing sicilian peaches samples aiming to identify and quantify eventually residues of Chlorpyriphos. LMR for chlorpyriphos in peaches, do not exceed 0.01 mg/kg.

MATERIAL AND METHODS

Gas chromatography tandem mass mass spectrometry (GC-MS) was used for the quantification and confirmation of Chlorpyrifos pesticide residues in peaches. The QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method was applied

for preparing samples. For the extraction procedure, 10.0 g of homogenized peach was weighed in a polypropylene tube. Afterwards, 10 mL acetonitrile was added and the sample vortexed for 1 min. After adding Supelco Citrate buffer mixture extraction, the sample was vortexed again for 1 min, and then it was centrifuged for 5 min at 3000 rpm. The extraction method was followed by a clean-up procedure by Supelco PSA dispersive solid-phase extraction (d-SPE).

The analyte was determined by ThermoFisher Trace 1310 gas chromatography- coupled with a triple quadrupole spectrometry TSQ-Quantum XLS by selected reaction monitoring (GC-MSMS-SRM) equipped with a TR-5MS 5% Phenyl Methyl Siloxan, 30 m x 250 µm x 0.25 µm column. The LoQ calculated is 0.005 mg/Kg.

Figure 1. Sicilian peaches.

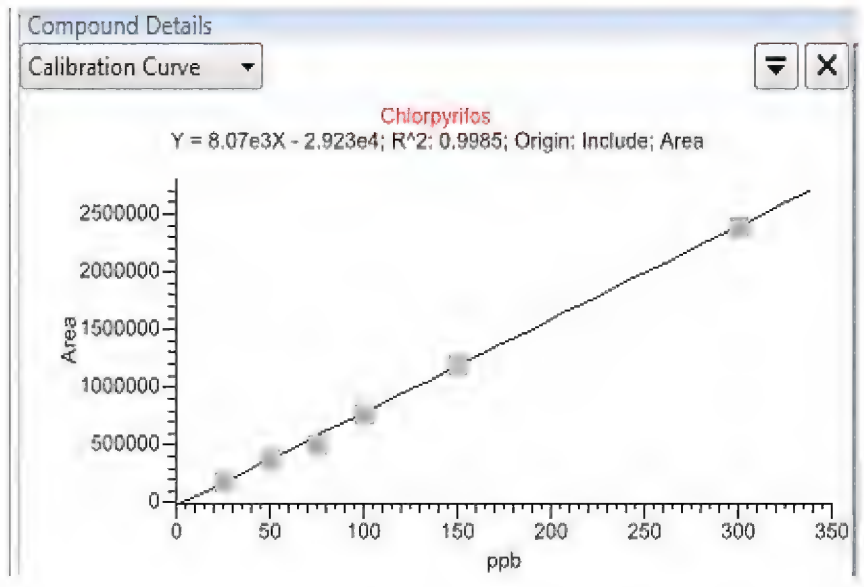

Figure 2. Calibration curve of the chlorpyrifos standard solutions expressed as ppb.

RESULTS AND DISCUSSION

150 samples of Sicilian peaches were collected in 3 years and analyzed for clorpyriphos. Clorpyriphos was revealed in 48 samples but under LMR value.3 samples was find a concentration over LMR (0.04 mg/Kg; 0.05 mg/Kg; 0.03 mg/Kg). Samples above the LMR are used as raw materials, so they will be processed by a dilution process that will make the product not hazardous to human health.

CONCLUSIONS

This study has demonstrated that QuEChERS is a rapid and reliable method to determination of chlorpyriphos from peaches.

The analysis carried out on peach samples confirmed that chlorpyrifos are widely used in Sicilian territory so that 34 percent of the samples were contaminated by the pesticide.

More attention should be paid to the use of Chlorpyrifos in order to reduce their detection levels.

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Biodiversity Journal, 2019, 10 (4): 493–494 https://doi.org/10.31396/Biodiv.Jour.2019.10.4.493.494
MONOGRAPH

First report on the presence of Alloxan in Bleached Flour by LC-MS/MS Method

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ABSTRACTS Alloxan is a ketone with a low molecular weight and neutral functional groups. In this work the presence of Alloxan in bread, pastry and cake bleached flour was investigate in order to verify possible risk for consumers related to the use of chemicals for flour bleaching.

KEY WORDS Alloxan; UHPLC MS/MS; toxicology.

Received 22.11.2019; accepted 10.12.2019; published online 20.12.2019
Proceedings of the 4th International Congress on Biodiversity "Man, Natural Habitats and Euro-Mediterranean Biodiversity", November 17th-19th, 2017 - Malta

INTRODUCTION

Alloxan is an oxygenated pyrimidine synthesized by uric acid oxidation that can be found in hydrated form. It was found to have toxicological effects on pancreatic b-cells leading to diabetogenic action, therefore (Pincus, 2013) is commonly employee for the development of Type-I Diabetes Mellitus in animal models (Carvalho et al., 2003). Alloxan also demonstrated to have a carcinogenic action in rats and fishes; furthermore, it can induce adenohypophysis cancer in mices (Suganuma et al., 1993). The aim of this work is to prove the presence of Alloxan in bleached flour, given the absence of data in literature on the relative toxicity of this molecule in bakery products. A selective UHPLC MS/MS method using precolumn derivatization was developed for the determination of Alloxan in flours.

MATERIAL AND METHODS

A total of 175 bleached flour samples were collected from manufacturers and local market of Sicily (Southern Italy). All the samples considered in this study were randomly collected by choosing different texture and size of granulation: 62 bread flour (slightly coarse), 55 pastry flour and 58 cake flour (smooth and fine), respectively. 2 g of the homogenized flour samples were weighed in a polypropylene centrifuge tube and spiked with 200 ml of Alloxan working solution at 10 mg mL 1 in Hydrochloridric acid 0.1 M to obtain a concen- tration of 1 mg kg 1. Every sample was mixed and allowed to rest for 15 min. Subsequently, 10 mL of hydrochloride acid 0.1M were added in the tube and then mixed for 1 min. The tube was vigorously centrifuged for 10 min at 3500 rpm; the supernatant was collected in a 50 mL polypropylene centrifuge tube that was filtered with filters of 0.45 mm. A 0.5 mL aliquot was added to 1.5 mL of 0.1% aqueous formic acid solution. This solution was spiked with 2 mL of o-phenylenedi- amine at 1 mg mL 1. After a gentle stirring, an aliquot of 1 mL was transferred into vials and set at the appropriate temperature of 25 C for 24 h, prior to LC-MS/MS analysis.

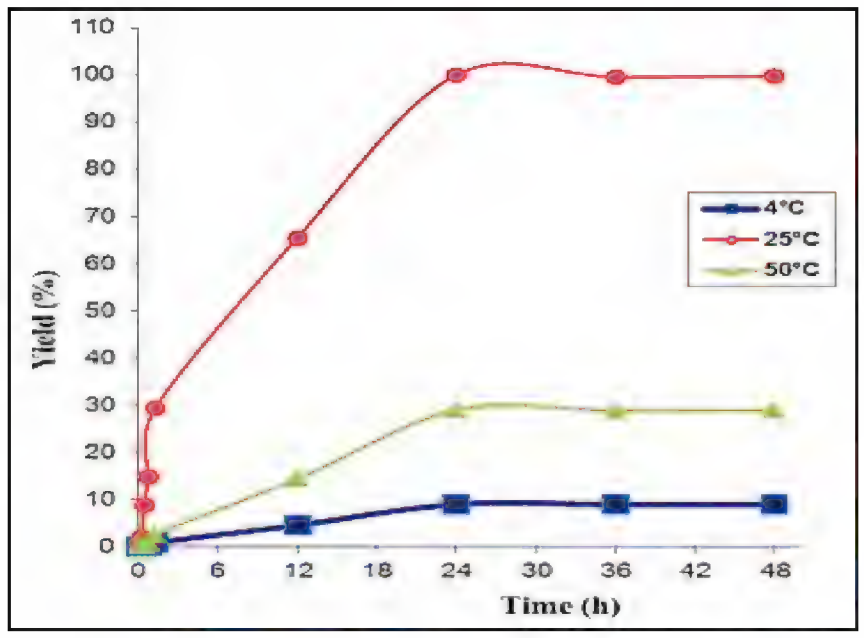

Figure 1. Graphic representation of the yield percentage of the derivatization reaction: kinetic of reaction studied as a function of the time at three different temperatures.

RESULTS AND DISCUSSION

The Alloxan fragmentation is difficult due to unstable transitions, so the ionization efficiency in ESI is low. The pre-column derivatization with o-phenylenediamine, which produces Alloxazine, has provided a better way to detect the analyte in question, due to a greater stability. Alloxazine is the result of reaction between a primary amine and carbonyl groups with formation of a product containing carbon nitrogen double bonds. The reaction is acid catalyzed by hydrochloric acid with elimination of two molecules of water. An alloxan solution was admixed with an excess of derivatizing agent (o-phenylenedi- amine), considering the stoichiometry as known and the yield as unknown. The instrumental results was collected at different time intervals: 5 min, 15 min, 30 min, 45 min, 90 min, 12 h, 24 h, 30 h and 48 h, in order to evaluating the reaction times. It was found an increasing trend of the yield percentage, up to a maximum value in the 24 h, following by a plateau in the next hours. The effect of the temperature on the yield was 4% at 4 C, 100% at 25 C and 29% at 50 C, respectively. Alloxan trace levels were found in 42 (24%) of the analyzed samples, with mean values of 0.95 ± 0.04 mg kg 1 and a range between 0.88 and 1.02 mg kg 1. Therefore, most of the cake flour are bleached in order to improve their baking per- formance and responding to a wide request for production. The use of chemical oxidizing agents and bleaches were developed to produce quick aging of wheat flour (48 h), instead the natural conditions that require several months. Chlorine gas used as bleaching agent may reacts with some proteins in the flour (including the gluten) producing Alloxan as a by-produc. High-gluten flours, such as the cake flour.

CONCLUSIONS

According to our knowledge (Vadlamudi et al., 1982; Galland & Senger, 1988; de Oliveira et al., 2005), this work represents the first report on the presence of Alloxan in cake bleached fl our, suggesting a potential risk for consumers due to the application of chlorine gas and other chemicals for baking cakes. The reported UHPLC e MS/MS method was found very sensitive and accurate for the determina tion of Alloxan in wheat fl our starting from 0.85 mg kg. The results obtained show that the fl our bleached with chlorine dioxide and chlorine gas may contain Alloxan as a minor product of a series of oxidation reactions. As a pilot stu dy, further studies are needed with a larger number of fl our samples, in order to understand the real risk for the consumers.

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Biodiversity Journal, 2019, 10 (4): 495–496 https://doi.org/10.31396/Biodiv.Jour.2019.10.4.495.496
MONOGRAPH

Impact of the "Anisakis C.Re.N.A." APP

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ABSTRACTS Anisakiasis is a zoonotical disease provoked by the nematodes belonged to the Anisakidae family. The trend of Anisakiasis cases increases continously, therefore a constant awareness of this topic is essential for the consumers' protection. An application for smartphones was carried out by the National Centre for Anisakiasis for this purpose. This work aimed at evaluate the impact of "Anisakis CReNA" app for consumers by the implementation of a survey. The results obtained revealed that the app developed has reached the goal established giving easy to understand and easy to consult information for the protection of consumers regarding the risks related to these parasites.

KEY WORDS *Anisakis*; National Reference Centre; health.

Received 22.11.2019; accepted 10.12.2019; published online 20.12.2019
Proceedings of the 4th International Congress on Biodiversity "Man, Natural Habitats and Euro-Mediterranean Biodiversity", November 17th-19th, 2017 - Malta

The application "Anisakis C.Re.N.A." was developed by the National Reference Centre for Anisakiasis (C.Re.N.A.) of Istituto Zooprofilattico Sperimentale della Sicilia, with the aim to divulging information about the health risk related to Anisakis parasites in fish products. There are several recommendations on the consumption and correct preparation of fish-based recipes and detailed information on their related diseases such as Anisakiasis with related photos and videos. It is possible to download it freely for the Android and iOS platforms.

Today, there are a lot of appllications which give a valuable contribution to ensure food safety giving the widespread use of smartphones or tablet. The app is proved to be very smart and easy because of its subdivision in topic icons. The homepage is divided as follow: informations about CReNA, *Anisakis*, Anisakiasis, normative and cooking recipes.

The digital devices that are use daily are the best tools for communication purpose concerning lifestyle areas, comprising cookery. For these reasons, the app contain delicious fish-based recipes with all the recommendations for consumers to ensure the maintenance of food safety. The work aimed to evaluate the impact of "Anisakis CReNA" app for consumers by the implementation of a survey.

A total of three hundred persons were contacted in supermarkets, fish street vendors and hotel management Schools of Sicily and primed to download and evaluate the app. Of these, 150 downloaded the app and evaluate it with a good feedback. A Monte Carlo test was conducted in order to have a clear estimation of the satisfaction grade subdivided by person category.

The test was carried out by R 2.1.4 software. The users that downloaded the *Anisakis* app were doctors, housewives and food sector operators. 80% of housewives showed a valuable interest on the *Anisakis*

topic, asking more information about the good practices of cooking fish products; the remaining 20% alerted themselves declaring to avoid the consumption of food products. Of these, over 90% were females between 50 and 60 years old. All the doctors gave a 100% positive feedback of the app highlighting the need to emphasize the information of the app to the consumers. 90% of the food sectors operators showed high interest to the information included in the app; the remaining 10% declared that the information of the app could be dangerous for the fish products sectors because are too alarmist and able to deviate the consumers. 94% of the users declared the app very useful, smart and easy. Finally, the results obtained revealed that the app developed has reached the goal established giving easy to understand and easy to consult information for the protection of consumers regarding the risks related to these parasites.

Biodiversity Journal, 2019, 10 (4): 497–498 https://doi.org/10.31396/Biodiv.Jour.2019.10.4.497.498
MONOGRAPH

Heavy metal toxicity and food contamination: lead, cadmium, and mercury determination on fish matrices from the FAO 37.2.2

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ABSTRACTS

Heavy metals can be essential, benefits and toxic. The 246 samples of swordfish, tuna and bivalve molluscs examined coming from the request of private individuals and at the request of the Control Authorities in order to carry out routine checks or inspections of food safety. All the elements were determined by an ICP-MS. The level of contamination of heavy metals in the fish species sampled in the Mediterranean Sea has been investigated as an indicator of water pollution. This study highlighted the presence of lead and cadmium, especially in swordfish samples, but all within the limits of law; mercury is absent in all samples. Top predators have higher levels of heavy metals than other species due to the bioaccumulation phenomenon.

KEY WORDS Heavy metals; marine top predators; contaminants; ICP-MS.

Received 22.11.2019; accepted 10.12.2019; published online 20.12.2019
Proceedings of the 4th International Congress on Biodiversity "Man, Natural Habitats and Euro-Mediterranean Biodiversity", November 17th-19th, 2017 - Malta

INTRODUCTION

The term "safety food" defines the measures and conditions necessary to control the dangers and ensure the suitability for human consumption of a food product as required by EC Regulation 852/2004. The heavy metal (lead, cadmium and mercury) contamination in fish food has reached a significant interest due to urbanization and industrialization. In recent years, this concept has undergone an evolution, as initially the only presence of parasites and pathogenic microorganisms was considered, while today it has been included the absence or presence of voluntarily added chemicals (additives and colorants), environmental pollutants and/or physical particles that could be harmful to human health by detecting contamination levels in the sampling area. Heavy metals are present in the soil, in the atmosphere, in water and in living organisms and are defined as: essential when in their absence do not allow the individuals of a certain species to grow and complete the biological cycle; benefits when their presence improves some aspects of the biological cycle; toxic when their biological effect is only unfavorable. The geographical position of Sicily, at the center of Mediterranean Sea,

ensures the obtained data are important informations about the contamination level which can be used to create a mapping of risk and its spread in the Italian seas having regards to fish species of Mediterranean Sea.

MATERIAL AND METHODS

The 246 samples of swordfish, tuna and bivalve molluscs examined have come to the Institute either at the request of private individuals and at the request of the Control Authorities in order to carry out routine checks or inspections of food safety. All the elements were determined by an ICP-MS (7700x series, Agilent Technologies, Santa Monica CA, USA) equipped with octopole reaction system (ORS3). The sample solutions were pumped by a peristaltic pump from tubes arranged on an autosampler ASX-500 Series (Agilent Technologies, Santa Monica (CA), USA) and then conducted on a quartz cyclonic spray chamber. A fast and efficient sample digestion was achieved by a microwave-assisted system Multiwave 3000 (Anton-Paar, Graz, Austria) equipped with a rotor for eight MF100 PTFE-TFM (poly-tetrafluoroethylene-tetrafluoroethylene) vessels.

RESULTS AND DISCUSSION

There are many works in the literature that deal with the processing of non-detectable data from a statistical-mathematical point of view in order to evaluate, in terms of conformity, the observation of greater variability for lead and cadmium concentration values in samples swordfish and tuna fish compared to samples of bivalve molluscs showing less variability (Bencko et al., 1995; Canli & Atli, 2003). For swordfish and tuna fish samples, concentrations of 0.28 mg/Kg were obtained with an average value of 0.09 mg/Kg against a maximum cadmium value of 0.10 mg/Kg with an average value of 0.015 mg/Kg and 0.029 mg/Kg. The maximum recorded mercury value is 0.10 mg/Kg and an average value of 0.003 mg/Kg and 0.061 mg/Kg. In bivalve mollusc samples, a maximum lead value of 0.27 mg/Kg was obtained with an average value of 0.02 mg/Kg and 0.10 mg/Kg. Mercury concentration values were all lower than 0.06 mg/Kg which is the quantification limit of the method used (<LoQ), while for the cadmium on 120 samples only two have a value above the quantification limit; 0.03 and 0.04 mg/Kg.

CONCLUSIONS

In this paper, the level of contamination of heavy metals (if existing) in the fish species sampled in the Mediterranean Sea (FAO 37.2.2 zone) has been investigated as an indicator of water pollution. This study highlighted the presence of lead and cadmium, especially in swordfish samples, but all within the limits of law; while mercury is absent in all the analyzed samples. This first evaluation shows a greater ability of swordfish to accumulate contaminants, as at the apex of the marine trout chain. Big predatory fish such as tuna and swordfish have higher levels of heavy metals than other species due to the bioaccumulation phenomenon and the trend towards long migrations. The maximum values and the mean concentrations recorded for lead and cadmium in the samples analyzed were all lower than the legal limits (see also Bencko et al., 1995; Canli & Atli, 2003).

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Biodiversity Journal, 2019, 10 (4): 499–516 https://doi.org/10.31396/Biodiv.Jour.2019.10.4.499.516
MONOGRAPH

Notes on some interesting species of Mollusca Gastropoda of the Monterosato collection from the Museum of Paleontology (Catania University, Italy)

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ABSTRACT In the year 2008, the then named Department of Geological Sciences of the University of Catania came into possession of malacological material belonging to Tommaso Di Maria, baron of Allery and marquis of Monterosato (Palermo, 1841–1927), an important malacologist specialized in the systematics of continental and mostly marine and fossil molluscs from the Mediterranean Sea and north east Atlantic. The small collection also includes interesting lots of shells belonging to other Sicilian naturalists such as the abbot Giuseppe Brugnone (Caltanissetta, Italy) and Pietro Calcara (Palermo, Italy). In this malacological collection, some interesting taxa little known and difficult taxonomic interpretations have been found. The rediscovery of the *Aghatina mandralisci* Calcara, 1840 and *Lachesis retifera* Brugnone, 1880 probable lectotypes, and the taxonomy of *Helix schwerzenbachi* Calcara, 1841 and *Helix cupani* Calcara, 1842 (syntypes) are discussed. Particularly, *A. mandralisci* is proposed as a synonym of *Allopeas gracilis* (Hutton, 1834), *H. schwerzenbachi* is proposed as a synonym of *Punctum* (*Punctum*) *pygmaeum* (Draparnaud, 1801), and H. *cupani* is proposed as a synonym of *Xerotricha conspurcata* (Draparnaud, 1801).

KEY WORDS Taxonomy; Mollusca; Gastropoda; Monterosato; malacological collection; Museum.

Received 12.07.2018; accepted 10.09.2018; published online 28.12.2019
Proceedings of the 4th International Congress on Biodiversity "Man, Natural Habitats and Euro-Mediterranean Biodiversity", November 17th-19th, 2017 - Malta

INTRODUCTION

Tommaso Di Maria, baron of Allery and marquis of Monterosato (Palermo June 27th, 1841 - ibidem, March 1st, 1927), was one of the most influential malacologists in the second half of the 19th century, specialized in the systematics of continental and mostly marine and fossil molluscs from the Mediterranean and north east Atlantic. He described a very large number of taxa, still valid today.

Much of the Monterosato specimens, since 1942, are hold in the malacological collections of the Museo Civico di Zoologia di Roma [= Township Zoological Museum of Rome (ZMR)] . These

collections have always been considered a great amount of information because they contain not only the specimens of Monterosato himself, but also acquired lots belonging to other contemporary malacologists, such as Giovanni Battista Adami, Nicola Tiberi, Giuseppe Antonio Brugnone, which in turn included shells belonging to Luigi Benoit, Ignazio Libassi and Pietro Calcara (Oliverio & Tringali, 2001).

In recent years, this important collection has undergone new studies and a complete list of new marine mollusc taxa introduced by Tommaso Allery Di Maria Marquis of Monterosato has been compiled (Oliverio & Tringali, 2001; Appolloni et al., 2018).

In February 2008, Ms. Laura Ryolo heir of the Marquis of Monterosato, donated library and malacological material belonging to him, to the then Department of Geological Sciences of the University of Catania. In the same year, the malacologist Stefano Palazzi cataloged the collection (Table 1, Fig. 1) allocated in the then Section of Oceanology and Paleoecology, now belonging to those of the today's Museum of Palaeontology of the Department of Biological, Geological and Environmental Sciences, University of Catania (Reitano, 2016).

In the present work, we will examine some species of this collection, which are poorly known and of particular taxonomic interest.

MATERIAL AND METHODS

The Monterosato collection kept at the Museum of Palaeontology of the University of Catania (MPCU) consists of 140 lots: 136 Gastropoda (marine, terrestrial, and freshwater recent and fossils species), 3 Bivalvia, and a mixed collection including molluscs and other taxonomic groups.

They arrived to the MPCU in a good state of preservation, inside a cardboard box. The shells with one or more labels were contained mostly inside variously shaped and sized glass vials and partly inside rectangular handmade cardboard boxes. The vials were closed with a cork stopper or cotton balls. Some of the most interesting shells were extracted from the original vial, to be photographed, and then placed back in a plastic box, to avoid harm from Byne's disease. The 140 lots were then placed inside cardboard boxes with plastic lids. Each box contains:

the original vial still closed, for lots containing uninspected shells;

the original vial, emptied of the shells, with its cap, both inside a zipped plastic bag, plus the shells in a plastic box;

the cm-sized shells, inside zipped plastic bags and/or relative cardboard boxes;

the original labels, inside plastic boxes when of compatible size, or inside zipped plastic bags;

a label bearing the words "Laura Ryolo Donation, Monterosato Collection, Sample n° (from 1 to 140)" and also the Latin name of the mollusc species, in square brackets when assigned by the reviser and finally the locality;

a label printed on a strip of paper, folded into three parts, with the header "University of Catania - Museum of Paleontology" bearing the inventory number of the collections on its lateral side. In the central part the complete inventory number of the Museum is reported, together with the progressive number of the Monterosato collection (as per the label mentioned above), the specific name (in square brackets when assigned by the reviser) followed by the number of specimens (in round brackets). The age, the location, and finally the initials of the reviser and the year of the revision (S[tefano] P[alazzi] 2008) are also reported.

In the present work are examined four lots we considered more interesting: 1136/13–33 *Bul*[*imus*] *mandraliscae*, 1173/13–33 *H*[*elix*] *schwerzenbachi*, 1178/13–33 *H*[*elix*] *cupaniana*, 1198/13-33 *Lachesis retifera* (Calcara, 1840a, b, 1841, 1842, 1845) (Figs. 2, 3).

Low magnification photos were acquired with a Canon EOS700D digital camera equipped with Tamron SP 60mm f/2 Di II LD Macro, in order to document the general features of the shells. Specimens were also examined uncoated under a Tescan Vega 2 LMU Scanning Electron Microscope in Low Vacuum modality to investigate tube micromorphology. All images were acquired at the Department of Biological, Geological and Environmental Sciences (University of Catania, Italy). The photographed specimens, although with some dirt of organic substances, have not undergone any cleaning, in order to avoid their damage.

ACRONYMS. MPCU: Museum of Paleontology, Catania University (Italy).

Numbers	Taxon revised by Palazzi, 2008	Age	Locality	Remarks
1060/13-33	[Bivalvia sp.]	Recent	Madéra	
1061/13-33	[*Alvania* spp.]	Recent	Madéra	
1062/13-33	[*Pusillina* and *Crisilla* spp.]	Recent	[Madéra]	
1063/13-33	[*Pusillina* sp.]	Recent	[Madéra]	
1064/13-33	*Raphitoma sandriana* var.	Recent	Ognina-Magnisi-Palermo	Ex coll. Brugnone
1065/13-33	*Nassa semistriata*	Pliocene	Savonese	
1066/13-33	*Nassa italica*	Pliocene	Castell'Arquato	Ex coll. Foresti
1067/13-33	[*Nassa*] *dertonensis*	Pliocene	[Castell'Arquato]	
1068/13-33	[*Raphitoma* sp.]	Recent	Pal[ermo]	
1069/13-33	*Helix aperta soluta-contraria*	Recent	Palermo	
1070/13-33	*H*[*elix*] *propemiralis*	Recent	Castelvetr[ano]	
1071/13-33	*P*[*ecten*] *jacob*[*aeus*] juv.	Recent	Palermo	
1072/13-33	*Helix glaberrima*	Recent	Madonie	
1073/13-33	*H*[*elix*] *rupestris*	Recent	England	Ex coll. Brugnone
1074/13-33	*H*[*elix*] *rupestris elata*	Recent	Palermo	Ex coll. Brugnone
1075/13-33	*H*[*elix*] *rupestris*	Recent	Oreto	Ex coll. Brugnone
1076/13-33	*Zonites nitidulus*	Recent	England	Ex coll. Brugnone
1077/13-33	[*Helix*] *gregaria*	Recent	Donnafugata e Ragusa	
1078/13-33	[*Monacha* sp.]	Recent	Toscana	Ex coll. Del Prete
1079/13-33	*Nassa cuvieri*	Pliocene	Altav[illa Milicia]	
1080/13-33	[*Retusa* sp.]	Recent	Magnisi	
1081/13-33	*Hel*[*ix*] *aspersa minor*	Recent	Ile de Re, Charente inf.	
1082/13-33	*Lithocerithium derelictum*	Recent	Tunisi lagoon	Ex coll. Pallary
1083/13-33	*Helix hiberna*	Recent	Gabriele	Ex coll. Brugnone
1084/13-33	[*Helix*] *onychina*	Recent	[sine loco]	
1085/13-33	*Helix cantiana*	Recent	York	
1086/13-33	[*Nassarius mutabilis*]	Recent	Pal[ermo]	
1087/13-33	[*Nassarius cuvierii*]	Recent	Pal[ermo]	
1088/13-33	*Helix libassiana*	Recent	[sine loco]	Name ms of Brugnone
1089/13-33	*Zonites excavatus*	Recent	England	Ex coll. Brugnone
1090/13-33	*Cl*[*ausilia*] *grimmeri avinensis*	Recent	Avinskogel	
1091/13-33	*Pupa superstructa*	Recent	Rutais (?)	
1092/13-33	[mainly Clausiliidae]	Olocene	Port Stewart, N. Ireland	
1093/13-33	*H*[*elix*] *sub(alpina?)*	Recent	Assisi	Ex coll. Del Prete
1094/13-33	[*Alvania* sp.]	Recent	Arenella [Palermo]	
1095/13-33	[*Pusillina* sp.]	Recent	Porto di Pal[ermo]	
1096/13-33	*Alvinia scabra*	Pleistocene	Milazzo	
1097/13-33	*Acinus hispidulus*	Recent	Corfù	Ex coll. Chaster
1098/13-33	[Collection of shells]	Recent	Cap Breton	
1099/13-33	[*Cochlicopa* sp.]	Recent	Castellam[mare, Trapani]	
1100/13-33	*Cochlicopa folliculus*	Recent	[sine loco]	Ex coll. Brugnone
1101/13-33	[*Ferussacia folliculus*]	Recent	S. Polo [Parma]	
1102/13-33	*Cochlicopa folliculus*	Recent	Brancaccio [Palermo]	Ex coll. Brugnone
1103/13-33	[*Ferussacia folliculus*]	Recent	Modica	

Table 1/1. Catalogue of the Monterosato malacological collection of the MPCU
(the content of the square brackets refers to the Stefano Palazzi's revision).

Numbers	Taxon revised by Palazzi, 2008	Age	Locality	Remarks
1104/13-33	*Acha*[*tina*] *petitiana*	Recent	Monte Cuccio et al.	Ex coll. Brugnone
1105/13-33	*Acha*[*tina*] *petitiana*	Recent	[sine loco]	Ex coll. Brugnone
1106/13-33	[*Cochlicopa*] *folliculus*	Recent	pr[esso] Palermo	
1107/13-33	*Ach*[*atina*] *gemmellariana*	Recent	Palermo	Ex coll. Brugnone
1108/13-33	*Ach*[*atina*] *rizzeana*	Recent	Palermo	Ex coll. Brugnone
1109/13-33	*Ach*[*atina*] *acicula*	Recent	England	Ex coll. Brugnone
1110/13-33	*Ach*[*atina*] *teres*	Recent	Palermo	Ex coll. Brugnone
1111/13-33	*Ach*[*atina*] *acicula*	Recent	Firenze	Ex coll. Brugnone
1112/13-33	*Ach*[*atina*] *actoniana*	Recent	Palermo	Ex coll. Brugnone
1113/13-33	*Ach*[*atina*] *pulchella*	Recent	Palermo	Ex coll. Brugnone
1114/13-33	*Ach*[*atina*] *hohenwarti*	Recent	Castelgoff[redo, Mantova]	Ex coll. Brugnone
1115/13-33	*Ach*[*atina*] *hohenwarti*	Recent	Magnisi e Bucche[ri]	Ex coll. Brugnone
1116/13-33	*Ach*[*atina*] *goniostila*	Recent	Palermo	Ex coll. Brugnone
1117/13-33	[*Ferussacia folliculus*]	Recent	Santa Cristina di Gela	
1118/13-33	[*Cecilioides* sp.]	Recent	Viareggio	
1119/13-33	[*Ferussacia folliculus*]	Recent	Varie località	Ex coll. Brugnone
1120/13-33	[*Caecum* sp.]	Recent		
1121/13-33	*Odostomia pusilla*	Pleistocene	Monte Pellegrino	Ex coll. Villa (?)
1122/13-33	*Alvinia clathrella*	Pleistocene	Milazzo	
1123/13-33	*Acme fusca*	Recent	Paler[mo]	Ex coll. Brugnone
1124/13-33	*Cochlicopa cylindracea*	Recent	Ponte di Corleone	Ex coll. Brugnone
1125/13-33	*Carychium minimum*	Recent	England	Ex coll. Brugnone
1126/13-33	*Cochlicopa lubrica*	Recent	Lentini	Ex coll. Brugnone
1127/13-33	*Acme fusca*	Recent	Ponte di Corleone	Ex coll. Brugnone
1128/13-33	*Cochlicopa tridentata*	Recent	England	
1129/13-33	[*Hohenwartia* sp.]	Recent	Orciano	
1130/13-33	*Zua collina subventricosa*	Recent	Siagne (?)	
1131/13-33	*Ach*[*atina*] *incerta*	Recent	Lipari	Ex coll. Brugnone
1132/13-33	*Cochlicopa lubrica*	Recent	Bozzano [Bolzano ?]	Ex coll. Brugnone
1133/13-33	*Carychium minimum*	Recent	Mondello	
1134/13-33	*Carychium tridentatum*	Recent	Palermo	
1135/13-33	*Ach*[*atina*] *petitiana*	Recent	Ognina et al.	
1136/13-33	*Bul*[*imus*] *mandraliscae*	Recent	Palermo	Ex coll. Brugnone
1137/13-33	*Carychium tridentatum*	Recent	Castelgoffredo [Mantova]	Ex coll. Brugnone
1138/13-33	*Cochlicopa lubrica*	Recent	Englande e Lesina	Ex coll. Brugnone
1139/13-33	*Ach*[*atina*] *bourguignatiana*	Recent	Monte Cuccio	Ex coll. Brugnone
1140/13-33	*P*[*omatias*] *margheritae*	Recent	Liguria	Settepassi [leg.] 1918
1141/13-33	[*Rissoina bruguierei*]	Recent	Adjim [Tunisia]	
1142/13-33	[*Rissoa* sp.]	Recent	Adjim [Tunisia]	
1143/13-33	[*Caecum subannulatum*]	Recent	Adjim [Tunisia]	
1144/13-33	[*Caecum* sp.]	Recent	Adjim [Tunisia]	
1145/13-33	[*Chrysallida* sp.]	Recent	Adjim [Tunisia]	
1146/13-33	[*Melanella praecurta*]	Recent	Adjim [Tunisia]	
1147/13-33	*Ti*[*ansiberus*] *subdentalis*	Recent	pr. di Marsala e di Mazzara	Ex coll. Brugnone, nomen never published

Table 1/2. Catalogue of the Monterosato malacological collection of the MPCU (the content of the square brackets refers to the Stefano Palazzi's revision).

Numbers	Taxon revised by Palazzi, 2008	Age	Locality	Remarks
1148/13-33	[*Theba pisana*] monstr. *scalaris*	Recent	Palermo Luglio	
1149/13-33	[*Theba pisana*] monstr. *perfecta*	Recent	pr. Palermo	
1150/13-33	*Cl*[*ausilia*] *byzantina*	Recent	Creta - Kambi pr. Psiloriti	
1151/13-33	*Cl*[*ausilia*] *(Delima) alschingeri*	Recent	Sine loco [Croatian endemism]	
1152/13-33	[*Helicidae* sp. juv.]	Recent	Brunnen Canton Schwyz	
1153/13-33	[*Marmorana platychela* monstr sinistral]	Recent	Sine loco	
1154/13-33	[*Clausilia*] *piceata*	Recent	flotté par le mer en Dalmatie provenant d'Italie	
1155/13-33	[*Ferussacia* sp.]	Recent	Favignana	
1156/13-33	*H*[*elix*] *rotundata* var. *albina*	Recent	Sine loco	Ex coll. Brugnone
1157/13-33	*Helix nemausensis*	Recent	Porte de Gentilly près Paris	
1158/13-33	*H*[*elix*] *subhispida* vel *calvula*	Recent	Palermo	Ex coll. Brugnone
1159/13-33	*Pleurotoma* sp.	?Pliocene	spiaggia di St. Raphael [Francia]	
1160/13-33	[*Eulima* cf. *glabra*]	Recent	Pal[ermo]	
1161/13-33	[*Rissoa* sp.]	Recent	Rabat [Marocco]	
1162/13-33	[*Vallonia* sp.]	Recent	Mondello	Ex coll. Brugnone
1163/13-33	[Helicidae sp.]	Recent	Madonie	Ex coll. Brugnone
1164/13-33	*Cl*[*ausilia*] *byzantina* var. *adspersa*	Recent	Creta Neokuri	
1165/13-33	*H*[*elix*] *vermiculata*	Recent	Linosa	
1166/13-33	*Helix aspersa*	Recent	Mauritius	Ex coll. Oberwimmer
1167/13-33	*Drillia modiola*	Miocene: Tortonian	S. Agata [Fossili - AL]	
1168/13-33	*H*[*elix*] *fulva*	Recent	Monte Cuccio et al.	Ex coll. Brugnone
1169/13-33	*Testacella haliotidea*	Recent	Parroco Gandolfo (Monte Cuccio)	Ex coll. Brugnone
1170/13-33	[*Chauvetia minima*] var. *albina*	Recent	Arenella	
1171/13-33	[*Oxychilus* sp.]	Recent	England	
1172/13-33	*Vitrina diaphana*	Recent	Castelgoffredo [Mantova]	Ex coll. Brugnone
1173/13-33	*H*[*elix*] *schwerzenbachi*	Recent	Oreto	Ex coll. Brugnone
1174/13-33	*Zonites alliarius*	Recent	England	Ex coll. Brugnone
1175/13-33	*H*[*elix*] *nitidosa*	Recent	Madonie	Ex coll. Brugnone
1176/13-33	*Cl*[*ausilia*] *(Delima) piceata*	Recent	Sine loco	
1177/13-33	*Daudebardia brevipes*	Recent	Ponte della Grazia	Ex coll. Brugnone
1178/13-33	*H*[*elix*] *cupaniana*	Recent	Orfanello presso Oreto	Ex coll. Brugnone
1179/13-33	*Zonites minutus*	Recent	Catania-Magnisi	Ex coll. Brugnone
1180/13-33	*H*[*elix*] *pulchella*	Recent	England	Ex coll. Brugnone
1181/13-33	*Claus*[*ilia*] *(Alopia) plumbea*	Recent	Brasso [Romania]	
1182/13-33	[*Alopia*] *haueri transitans*	Recent	Bratocia [Romania]	
1183/13-33	[*Cantareus apertus*] sinistral	Recent	Sine loco	
1184/13-33	[*Cantareus apertus*] *scalariforms*	Recent	Sine loco	
1185/13-33	[*Theba pisana*] *scalariforms*	Recent	Sine loco	
1186/13-33	[*Cantareus aspersus*] var. *flavida*	Recent	Capoterra [Sardegna]	
1187/13-33	[*Ercteila mazzullii*] sinistral	Recent	Sine loco	

Table 1/3. Catalogue of the Monterosato malacological collection of the MPCU (the content of the square brackets refers to the Stefano Palazzi's revision).

Numbers	Taxon revised by Palazzi, 2008	Age	Locality	Remarks.
1188/13-33	*H*[*elix*] *aspersa*	Recent	Sine loco	
1189/13-33	[*Marmorana muralis*]	Recent	Giardino Marchiello	
1190/13-33	*H*[*elix*] *globularis*	Pleistocene	[Monte] Pellegrino	Ex coll. Brugnone
1191/13-33	[*Nassa mutabilis*] var. *globulina*	Recent	Bastia [Corsica]	Ex coll. Locard
1192/13-33	*F*[*issurella*] *gibba* var. *radiata*	Recent	Sine loco	
1193/13-33	*Venericardia antiquata*	Recent	Sfax [Tunisia]	
1194/13-33	*Zonites obscuratus*	Recent	Monte Prana [Alpi Apuane]	Ex coll. Brugnone
1195/13-33	*H*[*yalinia*] *cellaria*	Recent	Madonie	
1196/13-33	[*Monacha* sp.]	Recent	Madonie	Ex coll. Brugnone
1197/13-33	*Helix aspersa*	Recent	Ponta Delgada Azzorre	
1198/13-33	*Lachesis retifera*	Plio-pleistocene	Giannettello	
1199/13-33	*Clausilia filfolensis*	Recent	Filfla [Malta]	Ex coll. Caruana Gatto. nomen ms never published

Table 1/4. Catalogue of the Monterosato malacological collection of the MPCU (the content of the square brackets refers to the Stefano Palazzi's revision).

Figure 1. The Monterosato malacological collection of the Museum of Palaeontology, Catania University (Italy).

MANDRALISCA ENRICO PIRAINO BARONE DI.

Catalogo dei molluschi terrestri e fluviatili delle Madonie e luoghi adiacenti. Palermo Stamperia Oretea 1840.

Nota di talune specie di molluschi terrestri e fluviatili di Sicilia. Palermo Estratto dal Giornale Letterario num 230. 1842.

PHYLIPPI R. A.

Enumeratio molluscorum Siciliae etc. Berolini 1836. Idem v. 2. Italis Savonum. 1844.

TESTA DOMENICO.

Due nuove specie di conchiglie rinvenute nei dintorni di Palermo.

SPIEGAZIONE DELLA TAVOLA.

Fig. 1. Helix Calcarae.
» 2. » Brocchi.
» 3. » Deshayesii.
» 4. » Scheverzenbachii.
» 5. » Cupani.
» 6. » Dibenedicti.
» 7. » Assorinensis.
» 8. » Nortoni.
» 9. » Usticensis.
» 10. Pupa contorta.
» 11. Bulimus cylindraceus.
» 12. » Mandralisci.
» 13. Limnaeus minimus.
» 14. Valvata Bocconi.
» 15. Paludina Mussonii.
» 16. » Porri.
» 17. » Salinasii.

(Memoria comunicata in dicembre 1844).

Figure 2. Caption on page 46 of the table attached to the work of Calcara (1845) where *Bulimus mandralisci*, *Helix schwerzenbachii*, and *Helix cupani,* found in the Monterosato malacological collection (MPCU), are drawn.

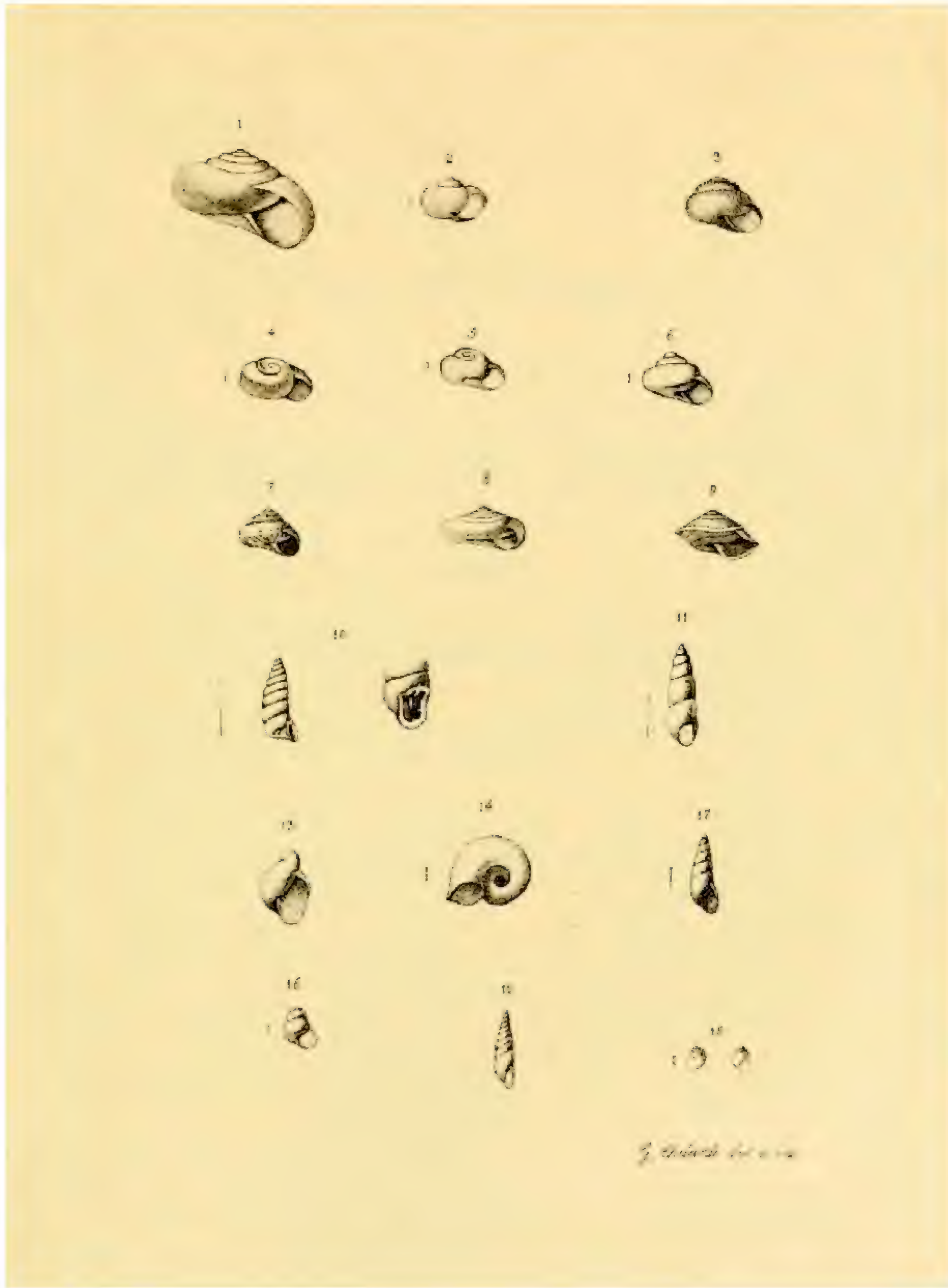


Figure 3. Table attached to the work of Calcara (1845) where *Bulimus mandralisci*, *Helix schwerzenbachii* and *Helix cupani,* found in the Monterosato malacological collection (MPCU), are drawn.

RESULTS

Aghatina mandralisci Calcara, 1840 (Figs. 4–7)

LOCUS TYPICUS. Calcara, 1840a: "*Conchiglia unica ritrovata dall'ornatissimo signor Testa nei contorni di Palermo ed esistente nella sua collezione*" [= surroundings of Palermo, Sicily, Italy].

DESCRIPTION. Calcara, 1840a: "*Testa cilindracea subfusiformis, subdiaphana flava apice obtuso, longitudinaliter tenuissime striata; anfractibus septem, convexo planis; sutura profunda divisis, apertura oblongo-ovata, labro simplici . Lunghezza 6 linee - Larghezza 2 1/6 - Altezza dell'apertura 2 linee*" .

Conical elongated slender whitish-opaque shell 9.50 mm high and 2.79 mm wide, consisting of seven whorls; last whorl and aperture high respectively the 49% and the 31% of the total shell. The protoconch is rounded with weak longitudinal striae, on the first whorl; a regular sutural crenulation starts with the second protoconch whorl. The teleoconch whorls are evenly rounded with a deep suture, which is crenulated by minute papillae. The surface of the whorls is covered by fine and dense axial striae with the widest curvature in the median region of each whorl. The last whorl is more flattened than the previous ones. The aperture is oblong-oval; the columella is straight and folded to close the umbilicus.

REMARKS. Calcara (1840a, b) refers to a single shell, on which he based the institution of this taxon, belonging to the collection of "*signor Domenico Testa*" of Palermo, and found by him "*nei contorni di Palermo* [in the surroundings of Palermo]". Calcara himself (1840b, in note 2) says to have never found it in so many researches and for this reason he considers it "*specie dubbiosa per la Sicilia* [a doubtful species for Sicily]".

Lancia (1853) considers the shell of *Bulimus mandralisci* to a "*var.* monstruosa" of *Hypnophila cylindracea* (Calcara, 1840). Based on this, Benoit (1862: 227) excludes *B. mandralisci* from the Sicilian species.

As reported by Cimino (2011), in Abbot Brugnone "*la passione per la malacologia fu alimentata dall'amicizia con l'affermato malacologo Domenico Testa che vantava una ricca collezione ritenuta allora superiore a tutte le altre e che metteva a disposizione dell'abate. Una collezione che, purtroppo, è andata distrutta durante la Spedizione dei Mille perché la casa del Testa era vicina al Palazzo Reale e fu distrutta da un incendio. Si salvarono solo alcuni rari pezzi che il Testa aveva scambiato con il Brugnone* [the passion for malacology was nourished by friendship with the affirmed malacologist Domenico Testa who boasted a rich collection considered superior to all the others at the time and which he made available to the abbot. A collection that, unfortunately, was destroyed during the Expedition of the Thousand because the Testa's home was close to the Royal Palace and was destroyed by fire. Only a few rare pieces were saved that Testa had exchanged for the Brugnone]".

Also Benoit (1882: 76) reports that Brugnone had the opportunity to take shells from the collection of Domenico Testa. The specimen we analyzed corresponds in size and morphological characters to the original description of *B. mandralisci* and could constitute its lectotype. This shell could then be passed from Testa to Brugnone and by the latter labeled with the name of *B. mandraliscae*. After the death of Brugnone (1884) the collection of the Nissen abbot was sold to the Marquis of Montesorato (Ryolo & Palazzi, 2008; Cimino, 2011).

We attribute the shell of *B. mandralisci* from the MPCU to the species *Allopeas gracilis* (Hutton, 1834) (Subulinidae). The species (graceful awlsnail) is widely distributed in subtropical and tropical areas of Asia, Africa, Australia, Polynesia, central-southern America, Caribbean islands and southeastern United States (Dundee, 1971; Capinera, 2017), also introduced in the Peninsula Arabica (Neubert, 1998) and Iraq (Naser, 2010). Although this species has been described from India, Neck (1976) and Auffenberg & Stange (1988) suggest that its origin is South America.

It is probable that the shell found in the surroundings of Palermo by Testa and described by Calcara (1840a) was introduced to Sicily with exotic plant species. It is more difficult to hypothesize whether this shell belonged to a living and acclimated population, albeit small or localized. Nowadays, little subulinids like *A. gracilis* have been found again in Palermo, inside the Botanical Garden (Roberto Viviano, *in verbis*) but, also in this case, without evidence of living populations.

Taking into account the above, we propose *B. mandralisci* as a younger synonym of *A. gracilis* (Hutton, 1834).

Lachesis retifera Brugnone, 1880 (Figs. 8–12)

LOCUS TYPICUS. Contrada "Giannettello" (today Giannittello), near Caltanissetta, Sicily, Italy.

DESCRIPTION. Brugnone, 1880: "*Conchiglia minuta, ovato-turrita, con apice ottuso e levigato, lunga mm. 6¼, larga mm. 2½: anfratti 7, leggermente convessi, separati da suture poco profonde, sottilmente reticolati: reticolo formato da linee poco elevate o costicine longitudinali e spirali, le une e le altre uguali tra loro ed ai loro intervalli, perlate nel loro incrociamento e formanti tante piccole areole incavate e quadrate come le maglie d'una rete; linne longitudinali rette, esistenti dalla seconda metà del primo giro, 24 nell'ultimo, quasi mancanti nella coda; linee spirali o transverse protratte dal secondo giro sino a tutta la coda, 13 nell'ultimo giro, 5 nel penultimo, 3 negli altri: apertura rotondata, coda cortissima, columella contorta; labbro destro acuto, internamente munito di 5 pieghe grossette, che cominciano sopra il canaletto ma non arrivano in alto; labbro sinistro tenue, distinto e continuo col destro*".

Shell buccinoid, 6.30 mm high, 2.6 mm wide, solid, with seven moderately convex whorls and a marked suture. Protoconch 750 µm in maximum diameter. The nucleous is smooth and 400 µm in diameter; the first whorl is markedly sculptured by axial ribs, narrower than the spaces separating them, where spiral cords are obvious at the intersection with the ribs generating small tubercles. The teleoconch ornamentations are spiral cords and axial ribs, both narrower than the interspaces. Interspaces between the ribs and cords form a rather uniform mesh, which form beaded tubercles. The first whorl consists of three spiral cords. In the last whorl there are 13 spiral cords, of which 4 from the penultimate whorl. The size of the cords is constant for almost the entire surface of the last whorl. Axial ribs on the last whorl 24 in number and prosocline. Last whorl and aperture high respectively the 60% and 42% of the total height of the shell. Outer lip enlarged, showing traces of reconstruction due to trauma. Inside the lip there are five weak denticles, the adapical one most evident; the abapical tooth forms the outer edge of the siphonal canal. White-yellowish color with some presumed traces of original coloring near the tubercles.

REMARKS. For a correct geological dating of this species, it should be considered that the presence of *Chlamys* (*Aequipecten*) *scabrella* (Lamarck, 1819) (Bivalvia Pectinidae) mentioned by Brugnone among the fauna found in Contrada Giannittello, would indicate it belong to the MPMU3 area (Monegatti & Raffi, 2001). In the geological map of Caltanissetta (http://www.isprambiente.gov.it/Media/carg/631-Caltanissetta-Enna/Foglio.html) near Contrada Giannittello (near Cozzo di Naro, SW of Caltanissetta) are outcropping at the bottom the marly clays of Geracello (Upper Pliocene) and towards the other the sands of Lannari (Upper Pliocene-Lower Pleistocene?). Since the exact collection point of the fossil is unknown, we attribute its age, as a precaution, to the Upper Pliocene-Lower Pleistocene.

The taxonomic aspects relating to *Chauvetia retifera* have already been extensively discussed (Micali, 1998; Tringali, 2001). In the present work, the taxon *Chauvetia retifera* is considered applicable only to the fossil species treated here. Therefore we believe that the name *Chauvetia elongata* F. Nordsieck & Garcia-Talavera, 1979 is available and applicable for the living species, both in the Atlantic and Mediterranean (Micali, 1998; Gofas & Oliver, 2010), unlike what is reported in MolluscaBase (www.molluscabase.org/aphia.php?p=taxdetails&id=138890; last consultation 21/12/2019). As already believed by Tringali (2001) the taxa described by Monterosato (1889) are not available, having been published as synonyms for *Lachesis retifera* (ICZN, 2019: Art. 11.6), and as varieties are not available (ICZN, 2019: Art. 45). At present *Chauvetia retifera* is not known for any other Plio-Pleistocene deposit in the Mediterranean area.

The species differs in general from *Chauvetia elongata* in having a smaller shell and less slender profile (see also Table 2).

Helix schwerzenbachii Calcara, 1841 (Figs. 13–15)

LOCUS TYPICUS. Calcara, 1841: "*Si rinviene comunemente attaccata sopra le pietre della contrada di Bellolampo e come ancora nelle vicinanze delle sponde dell'Oreto* [near Palermo]".

DESCRIPTION. Calcara, 1841: "*Testa minuta, orbiculata, tenui, pellucida, glabra, corneo fulva, late*

	Chauvetia retifera	*Chauvetia elongata*
Protoconch	Maximum diameter 750 µm with a nucleus 400 µm wide	Maximum diameter 850 µm with a nucleus 450 µm wide
Teleoconch	Spiral cords narrower than the interspaces	Spiral cords twice the size of the interspaces
	Axial ribs narrower than the interspaces, which form beads at the intersection with the spiral cords	Axial ribs of the same size of interspaces, slightly elevated which form quadrangular nodules at the intersection with the spiral cords
	Whorls moderately convex that mark a deep suture	Whorls little convex that mark a canaliculate suture
	24 axial ribs in the last whorl	20-24 axial ribs in the last whorl
	13 spiral cords on the last whorl, four of which came from the penultimate whorl	17-18 spiral cords on the last whorl, five of which came from the penultimate whorl
	Last whorl high 60% of the total height of the shell	Last whorl high 56-58% of the total height of the shell

Table 2. Morphological differences between *Chauvetia retifera* and *C. elongata*.

umbilicata; anfractibus 3 convexis, ultimo magno inflato; apertura rotundata patula, labro simplici, acuto. Diametro 1/3 di linea [equivalent to about 0.8 mm]".

Shell discoidal in shape and very small, light horny brown and shiny, teleoconch consisting of three convex whorls; external surface with dense and regular ribs, on the ventral side around the umbilicus about 20 rows of very prominent, blunt spiral ribs crossed by axial ribs, followed by an area with only axial sculpture towards the outer edge of the body whorl; aperture rounded, margin thin (but broken), umbilicus wide.

REMARKS. According to Calcara (1841) this taxon differs from the similar "*Helix*" *pygmaea* Draparnaud, 1801 in having a shell one third smaller, for a smaller number of whorls, for the orbicular shape and for having a wider umbilicus.

The species was accepted by Aradas & Maggiore (1841: 60) and Calcara (1845: 17) but most of the later authors considered the taxon *H. schwerzenbachii* Calcara, 1841 doubtful and based on juvenile specimens: Philippi (1844: 218), Pfeiffer L. (1848: 218; 1853: 272; 1868: 492), Kobelt (1875: 10), Tryon (1887: 30), Taylor (1909: 160).

Benoit (1859: 144–145, Pl. 4, f. 8) describes the species of Calcara sub *H. schwerzenbachiana* and he too compares it with "Helix pygmoea". However, the description of Benoit (1859) for some characters is different from that of Calcara. In particular, Benoit (1859) describes the last angled and dilated whorl, the rounded lunar aperture, the smallest umbilicus and the presence of a lamella that originates between the two edges of the peristome and continues inside the coil. These characteristics lead us to believe that *H. schwerzenbachiana* Benoit, 1859 is based on juvenile specimens of a different species from that of Calcara (1841), probably *Lauria cylindracea* (Da Costa, 1778).

The two specimens preserved in the Monterosato collection of the MPCU are *juveniles* since their heights are respectively 1.03 mm (1173/13-33a) and 1.027 mm (1173/13-33b). Both show apex and peristome heavily damaged. The shape of the shell and the ornamentation of these specimens lead us to classify them as *Punctum* (*Punctum*) *pygmaeum* (Draparnaud, 1801), a species with a wide Holarctic distribution, present all throughout Italy (Manganelli et al., 1995) and mainly linked to undergrowth environments of wooded areas.

These two specimens are probably syntypes of the species described by Calcara because, in the label inserted in this lot, the abbot Brugnone reported the wording "*avuta dall'autore* [had by the

author]", moreover Brugnone bought shells directly from Calcara (Cimino, 2011), which subsequently merged in the Monterosato collection (Ryolo & Palazzi, 2008).

In consideration of the above, *Helix schwerzenbachii* is proposed here as a synonym of *Punctum* (*Punctum*) *pygmaeum* (Draparnaud, 1801).

Helix cupani Calcara, 1842

LOCUS TYPICUS. Calcara, 1842: "*Trovasi nelle sponde di fiume Oreto vicino Ponterrotto*" [near Palermo, Sicily, Italy].

DESCRIPTION. Calcara, 1842: "*testa parva, orbicolato-depressa, corneo-fulva, inferne convexa late umbilicata; anfractibus 2 a suturis impresis, superficie puctulato-scabra, subpilosa, labro tenui, simplici. Diametro ½ linea* [equivalent to about 1.15 mm]".

Shell dextral, very small, pale light brown, globose; 2–2.1 convex whorls. The protoconch, about 1.5 whorls, is regular spirally striated, sometimes, with faint ribs more evident near the sutura. On the ventral side, around the umbilicus, spiral striae are weaker, and shorts ribs have a concentric shape. The teleconch whorl is globose with irregular ribs. The surface of the shell is covered by irregular periostracal hairs. Aperture rounded, margin thin slightly reflected towards the umbilicus.

REMARKS. From a nomenclatural point of view, Benoit (1859: 146) re-describes and depicts the Calcara's species by amending the name *H. cupani* to *H. cupaniana* and citing the original spelling in the text. Subsequently, Pfeiffer (1868: 431–432) places *H. cupaniana* Benoit, 1859 in synonymy with *H. cupani* Calcara, 1842. However, the name *H. cupaniana* attributed to Calcara (1842) continued to be used by most of subsequent authors: Benoit (1875: 138; 1882: 40), Westerlund (1876: 39; 1890: 3, 49), Reinhard (1877: 285), Kobelt (1877: 285), Pilsbry (1894: 47), Taylor (1909: 180), Ruhoff (1980: 227).

Although the taxon *H. cupani* Calcara, 1842 was used by some authors of the 1800s, including Aradas & Maggiore (1841: 60), Calcara (1845: 18) and Pfeiffer (1848: 424), the taxon *H. cupaniana* has remained in predominant use, attributed to the original author Calcara (1842). Therefore, it can be considered a justified amendment of *Helix cupani* Calcara, 1842 (I.C.Z.N. Art. 33.2.3.1.).

From a taxonomic point of view, *H. cupaniana* Calcara, 1842 was prevalently considered a doubtful species, with a description based on juvenile specimens. Philippi (1844: 218) hypothesizes that *H. cupani* may be based on juvenile specimens of *Xerotricha apicina* (Lamarck, 1822), as also shared by Pilsbry (1894: 254). Benoit (1859: 146, Pl. 4, f. 9) accepts the species of Calcara (1842) and publishes a description and drawing compatible with young specimens of *Xerotricha* Monterosato, 1892. However, figures 9 and 10 of Benoit's Plate 4 (1859) are reversed (see Kobelt, 1877: 285). Reinhard (1877: 285) and Kobelt (1877: 285), on the basis of specimens from the Berlin Museum, place *H. cupaniana* in synonymy of *Discus* (*Gonyodiscus*) *rotundatus rotundatus* (O.F. Müller, 1774). This synonymy was followed by later authors as Westerlund (1890: 3, 49), Pilsbry (1894: 46–47) and Taylor (1909: 180).

The lot of the MPCU is made up of 43 rather uniform specimens in terms of size and morphological characteristics. These characters allow us to attribute these shells to the species *Xerotricha conspurcata* (Draparnaud, 1801).

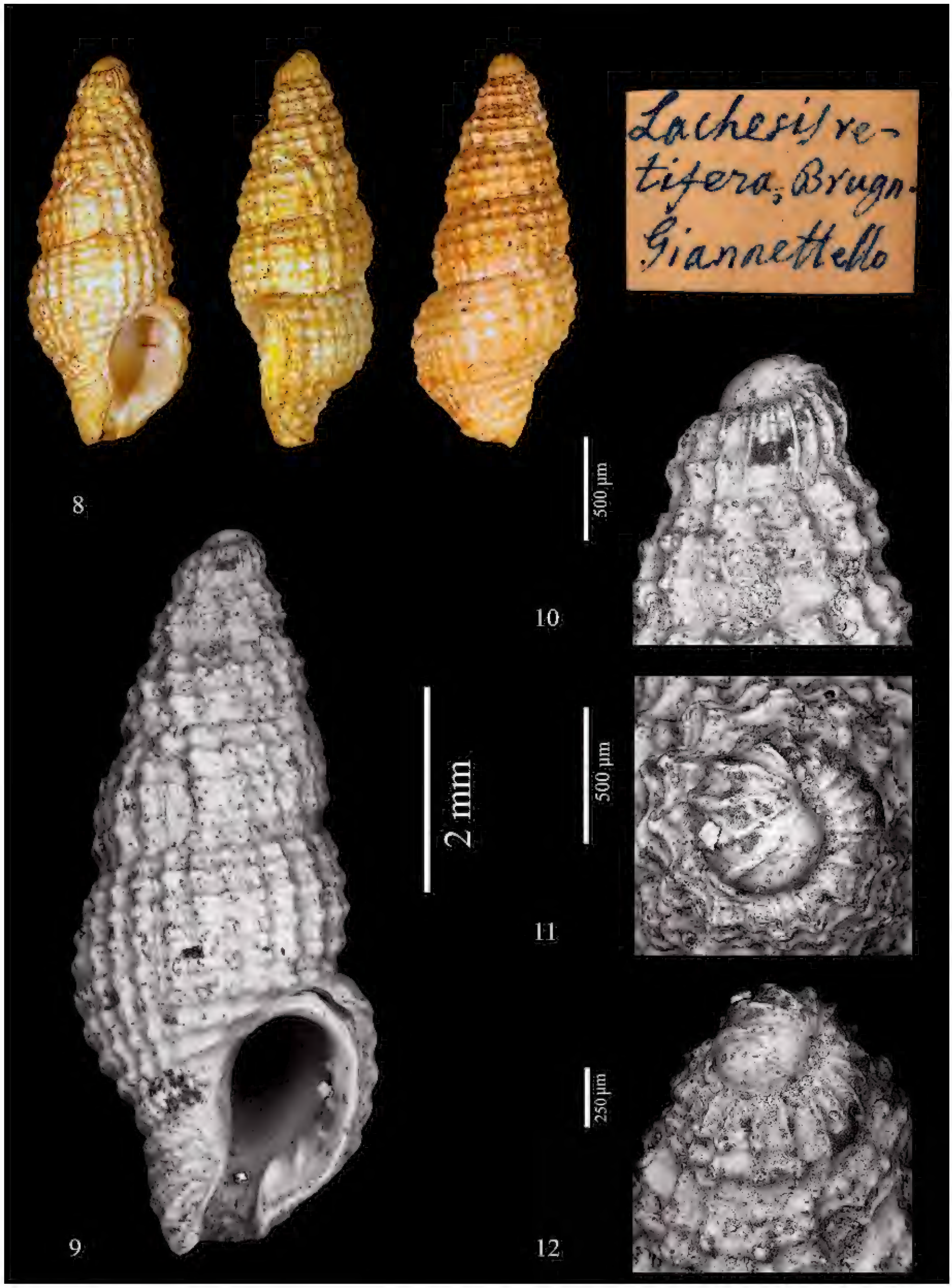
The original description of *H. cupaniana* Calcara, 1842 shows the term "*subpilosa*", and therefore corresponds better to the specimens of *X. conspurcata* of the MPCU, rather than to the specimens of *D. rotundatus* of the Berlin Museum. Furthermore, the locality "Orfanello near Oreto" written on the label of the MPCU specimens is very close to the type locality of *H. cupaniana*: "banks of the Oreto river near Ponterotto".

Considering that, Brugnone purchased shells directly from Calcara (Cimino, 2011) the specimens of the MPCU, ex Monterosato collection, ex Brugnone collection can be considered syntypes.

In consideration of the above, *Helix cupaniana* Calcara, 1842 is proposed here as a synonym of *Xerotricha conspurcata*.

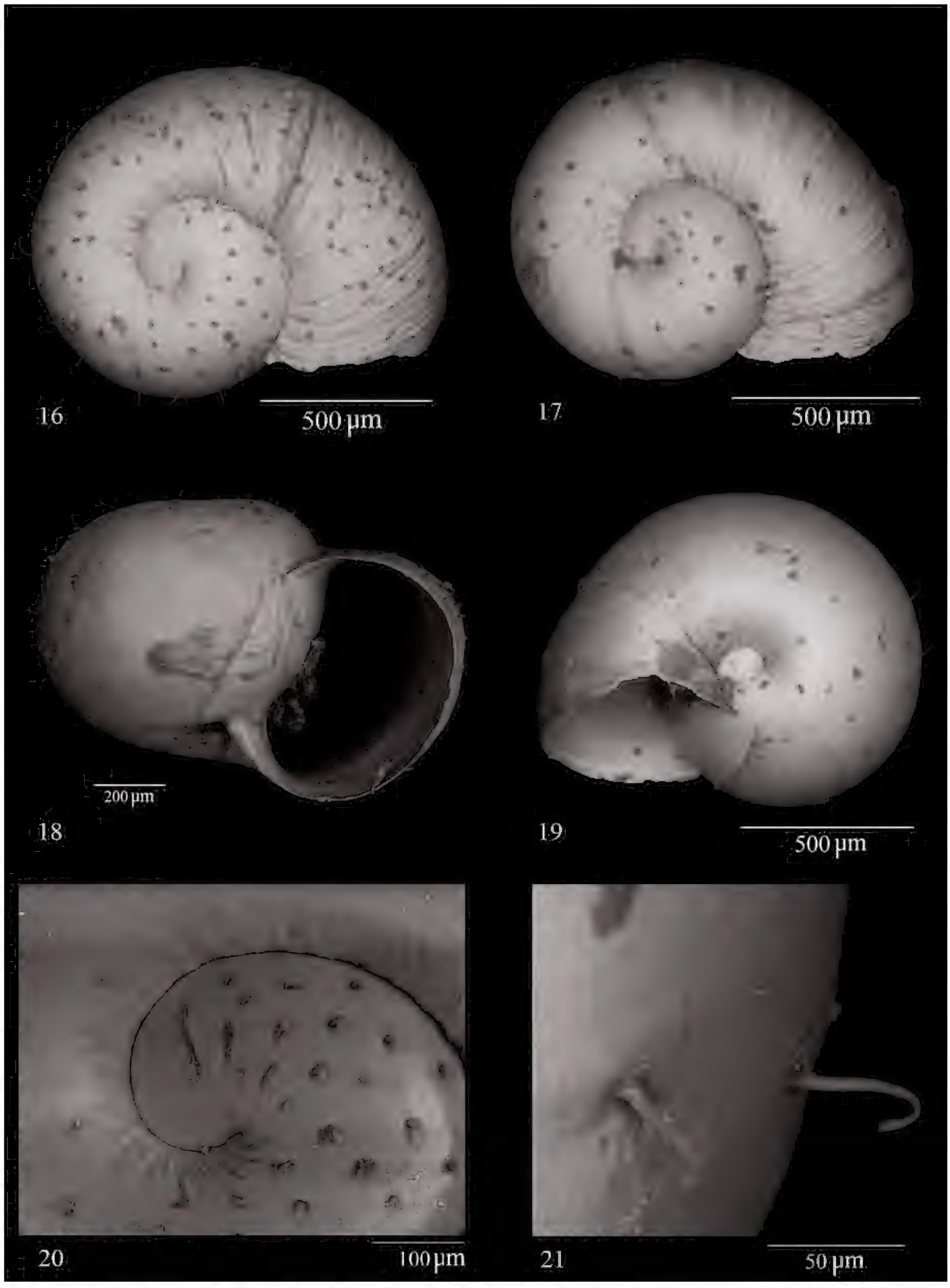
Bourguignat (1878?) established the new genus *Allerya* for three species of small molluscs from Sicily: *A. monterosati* n. sp., *A. brocchi* (Calcara, 1842) and *A. cupanii* (Calcara, 1842). It is likely that the description of *A. monterosati* and *A. brocchi*, as for *H. cupaniana*, was based on juvenile specimens of already described species. Thus, further studies are necessary for a correct taxonomic interpretation of both *A. monterosati* and *H. brocchi* and of the genus *Allerya*.

Figures 4–7. *Aghatina mandralisci* Calcara, 1840 from Monterosato malacological collection (MPCU). Figs. 4, 5: shell and original label. Figs. 6, 7: protoconch.



Figures 8–12. *Lachesis retifera* (Brugnone, 1880) from Monterosato malacological collection (MPCU). Figs. 8, 9: shell and original label. Figs. 10–12: protoconch.

Figures 13–15. *Helix schwerzenbachii* Calcara, 1841 from Monterosato malacological collection (MPCU). Fig. 13: shells 1173/13–33a and 1173/13–33b and original label. Fig. 14: shell 1173/13–33a. Fig. 15: shell 1173/13–33b.



Figures 16–21. *Helix cupani* Calcara, 1842 from Monterosato malacological collection (MPCU). Fig. 16: shell 1198/13–33a. Fig. 17: shell 1198/13–33b. Fig. 18: shell 1198/13–33c. Fig. 19: shell 1198/13–33d. Fig. 20: shell 1198/13–33e; protoconch nucleus. Fig. 21: shell 1198/13–33e, detail of the periostracal hairs and microsculpture.

Figure 22. *Helix cupani* Calcara, 1842 from Monterosato malacological collection (MPCU), shells and original label.

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Biodiversity Journal, 2019, 10 (4): 517–526 https://doi.org/10.31396/Biodiv.Jour.2019.10.2.517.526
MONOGRAPH

Molecular studies on the genus *Muticaria* Lindholm, 1925 (Pulmonata Clausiliidae) from the Maltese Islands

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ABSTRACT The present study has been carried out with focus on *Muticaria macrostoma* group from the Maltese Islands to characterize and define, from a molecular standpoint, their identity and relationships with topotypical Sicilian *Muticaria* (i.e. *M. syracusana*, *M. neuteboomi*, *M. cyclopica* and *M. brancatoi*). Molecular study included amplification of 16S rDNA (ca. 300 bp) and COI (ca. 700 bp) gene partial sequences which were used for single and combined gene analysis by Bayesian Inference to achieve the phylogenetic reconstructions with the highest posterior probabilities. Obtained results showed that, within *M. macrostoma* group, the taxa *mamotica* and *oscitans* can be elevated to the specific rank, thus bringing to three the Maltese *Muticaria* species, i.e. *M. macrostoma*, *M. mamotica*, and *M. oscitans*; whereas *scalaris* may be considered a subspecies, or even a synonim. Present findings confirmed the validity of the Sicilian species *M. syracusana*, *M. neuteboomi*, *M. cyclopica* and *M. brancatoi*. Furthermore, the populations of the Sicilian and Maltese *Muticaria* seem to belong to two different levels of differentiation. Finally, we have also examined some *Lampedusa* populations but the position of this genus still remains to be clarified. In particular, it is confirmed that *Lampedusa* and *Muticaria* are different genera, but at present, the relations within the *Lampedusa* group need further studies to be analysed in details.

KEY WORDS *Muticaria macrostoma* group; Malta; Phylogenetic reconstructions; 16S rDNA; COI.

Received 25.07.2018; accepted 03.10.2018; published online 28.12.2019
Proceedings of the 4th International Congress on Biodiversity "Man, Natural Habitats and Euro-Mediterranean Biodiversity", November, 17th-19th 2017 - Malta

INTRODUCTION

The genus *Muticaria* Lindholm, 1925 (Clausiliidae Alopiinae Medorini) comprises xeroresistant and calcicolous molluscs, distributed in Central-eastern and South-eastern Sicily and Maltese Islands. At present, four *Muticaria* species can be found on the Sicilian territory: *M. syracusana* (Philippi, 1836), *M. neuteboomi* Beckmann, 1990, *M. brancatoi* Colomba, Reitano, Liberto, Giglio, Gregorini et Sparacio, 2012 and *M. cyclopica* Liberto, Reitano, Giglio, Colomba et Sparacio, 2016; while

only *M. macrostoma* Cantraine, 1835 is reported for the Maltese Islands (Bank, 2017; Bank & Neubert, 2019).

As far as concerns the distribution of Sicilian *Muticaria* species, *M. syracusana,* locus typicus Siracusa (Philippi, 1836), occurs along the entire coast embracing Siracusa province; *M. neuteboomi*, locus typicus Cave d'Ispica, Ragusa province (Beckmann, 1990), inhabits a quite broad area including Siracusa, Ragusa, Caltanissetta and Catania provinces, and generally occurs at higher altitudes than those reported for *M. syracusana*; *M. brancatoi* is known only for the localities of description, i.e. Cugnolungo (type locality), Spinagallo, Vallone Moscasanti (Siracusa) (Colomba et al., 2012); and, finally, *M. cyclopica,* type locality Castello di Eurialo (Epipoli, Siracusa), is currently reported only for Epipoli, a hill about 150 m high, very close to the modern city of Siracusa (Liberto et al., 2016).

On the other hand, the Maltese species includes morphologically different populations that have been variously considered different species, or subspecies or simple morphs (see also Holyoak, 1986; Beckmann, 1992; Giusti et al., 1995; Nordsieck, 2007). Currently, one species with four subspecies is accepted (Bank, 2017; Bank & Neubert, 2019):

M. macrostoma macrostoma, locus typicus Malta (Cantraine, 1835), reported for Gozo, Comino, Cominotto and Malta;

M. macrostoma scalaris (L. Pfeiffer, 1850), locus typicus: Malta (L. Pfeiffer, 1850), inhabiting a very limited area on the Northwestern coast of Malta (Tal-Blata, Mistra Bay = St. Paul's Bay);

M. macrostoma oscitans (Charpentier, 1852), locus typicus: Malta (Charpentier, 1852), reported for Gozo and Malta;

M. macrostoma mamotica (Gulia, 1861), locus typicus: "*in insula Gaulos*" (Gulia, 1861), occurring in a very limited area on the Munxar side of Xlendi Valley in Gozo.

Numerous studies have been conducted on Maltese *Muticaria*, especially on the morphology of the shell and the genital organs (see Giusti et al., 1995 and cited bibliography), with often conflicting results on the taxonomic interpretation, also due to the presence of hybridization phenomena between some populations (Giusti et al. 1995; Cilia et al., 2012).

Taking into account available literature data and results obtained in previous studies carried out by this research team (Colomba et al., 2010; 2012; Liberto et al., 2016), we decided to further investigate on this group (Colomba et al., 2017). In particular, we focused on *M. macrostoma* from the Maltese Islands in order to characterize and define, from a molecular standpoint, their taxonomic status and the relationships with Sicilian *Muticaria*. In this work on *Muticaria*, we also included *Lampedusa imitatrix* (O. Boettger, 1879) specimens from Malta to study the relationships between these two genera that share part of the same area in the islands of the Sicilian Channel. Indeed, the genus *Lampedusa* O. Boettger, 1877 is distributed also in the Pelagie Islands (Sicilian Channel) with *L. lopadusae lopadusae* (Calcara, 1846) from Lampedusa and *L. lopadusae nodulosa* (Monterosato, 1892) from Lampione.

Lampedusa and *Muticaria* were also investigated using genetic data (sequencing of a fragment of the mitochondrial large ribosomal subunit 16S rRNA, and the nuclear internal transcriber spacer 1, ITS-1 rRNA) with a study available online as a bioRxiv preprint (Fiorentino et al., 2017, http://dx.doi.org/10.1101/208348).

Our results, compared also with available data, will be employed to achieve a better understanding of the speciation and dispersal phenomena of the populations of these interesting alopiine clausiliids and provide useful indications for their taxonomy.

MATERIAL AND METHODS

Samples and Collection sites

Two to five specimens per each population were employed for the study. Representatives of all subspecies of *M. macrostoma* sampled in different locality on the Maltese Islands, in addition to the topotypical populations of Sicilian *Muticaria* species, were analysed, along with specimens of *Lampedusa imitatrix* from Malta. Data on samples and collection sites, including the acronyms of the different examined populations, localities and GenBank Accession Numbers are reported in Table 1.

As seen in Table 1, in some cases where the specimens initial identification was not certain (ei-

ther at the subspecific or specific level) the samples were labelled separately and differently, i.e. *macrostoma* x *oscitans* (see also acronyms). Each collection site or locality is named in the original languages (i.e. Italian or Maltese).

DNA extraction

Samples were stored separately at -20 °C in test tubes. For each individual, a piece of foot tissue was used for total DNA extraction (by Wizard Genomic DNA Purification Kit, Promega). Voucher specimens were stored in the laboratory of Cytogenetics and Molecular Biology (University of Urbino, via Maggetti 22). Fragments of 16S rDNA (251–297 bp): and COI (529–660 bp) sequences were amplified using the primer pairs: MED16F/R (forward: 5'-ACTGTGCAAAGGTAGCATAA-3'/reverse: 5'-CCAACATCGAGGTCACAA-3') designed by Colomba and LCO_1490/HCO2198 as in Folmer et al. (1994). PCR cycles were as follows: 95 °C for 5 min; 95 °C for 1 min, 55 °C for 1 min, 72 °C for 1 min (30 cycles); 72 °C for 5 min (16S rDNA); 95 °C for 5 min; 95 °C for 1 min, 42 °C for 1 min, 72 °C for 1 min (35 cycles); 72 °C for 5 min (COI). To remove primers and unincorporated nucleotides, the amplified products were purified by the Wizard SV gel and PCR Clean-up kit (Promega). Sequencing of the purified PCR products was carried out using automated DNA sequencers at Eurofins MWG Operon (Germany). GenBank Accession Numbers for all sequences generated in this study are listed in Table 1. Homologous sequences of *Clausilia bidentata* (Strøm, 1765) (AF012082; JX911288), *Medora garganensis* (A.J. Wagner, 1918) (KC833909; KC853248), *Arianta arbustorum* (Linnaeus, 1758) (JF717810; MF140994) and *Massylea vermiculata* (O.F. Müller,

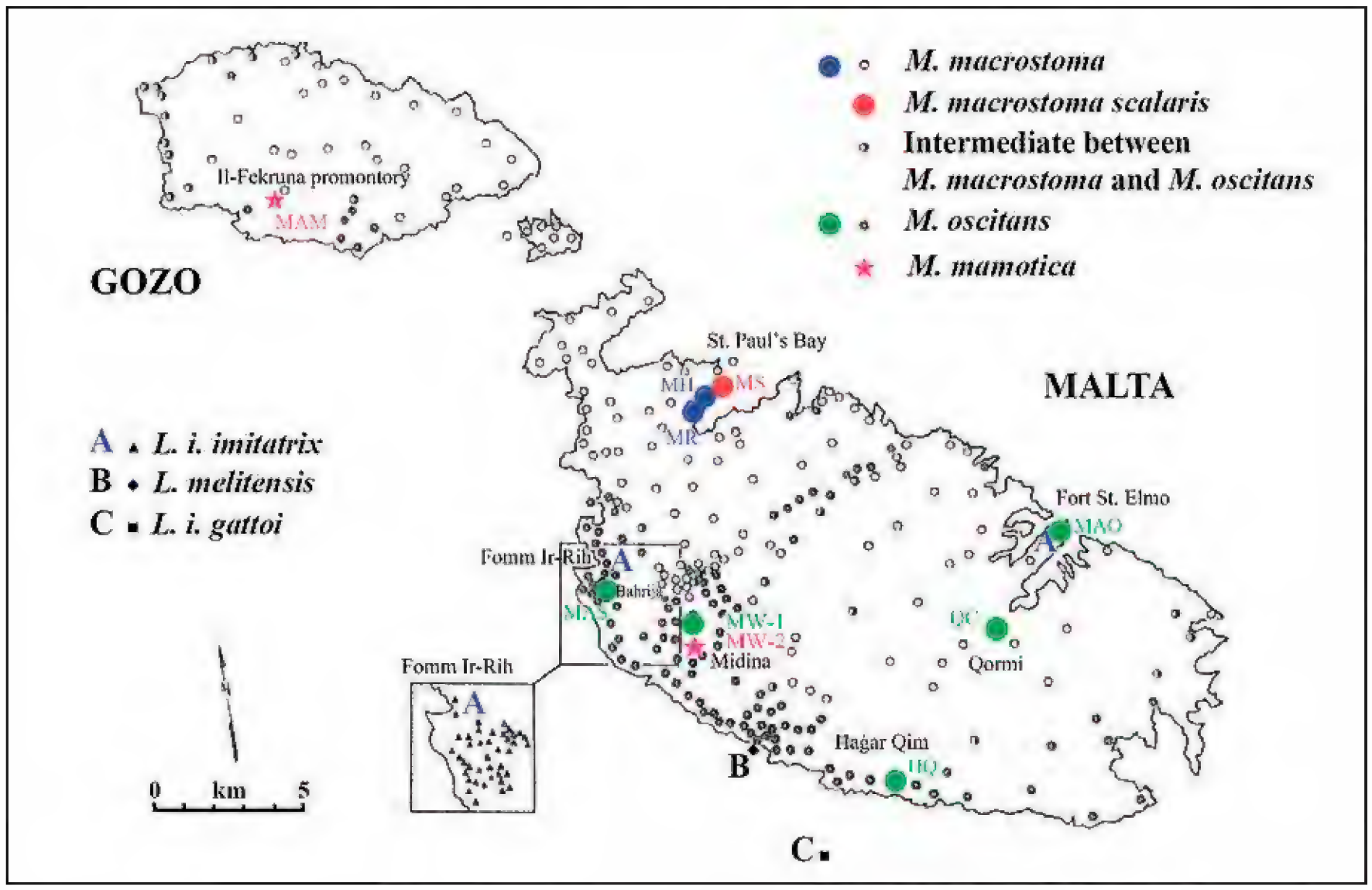

Figure 1. Distribution map of *Muticaria* and *Lampedusa* in the Maltese Islands (Holyoak, 1986 modified): white dots: distribution of *M. macrostoma macrostoma*; blue dots: sampling localities of *M. macrostoma macrostoma*; red dot: distribution and sampling localities of *M. macrostoma scalaris*; white and black dots: distribution of morphologically intermediate populations between *M. macrostoma macrostoma* and *M. macrostoma oscitans*; black dots: distribution of *M. macrostoma oscitans*; green dots: sampling locaties of *M. macrostoma oscitans*; purple star: distribution and sampling locality of *M. macrostoma mamotica*; triangles: distribution of *L. imitatrix imitatrix*; blue A: sampling localities of *L. imitatrix imitatrix*; rhombus: distribution of *L. melitensis*; square: distribution of *L. imitatrix gattoi*.

Taxon (initial classification)	Collection site	Voucher label	Coordinates	Species (revised classification)	GenBank Accession Number
M. m. macrostoma 1	St. Paul's Bay, Mistra, Malta	MH	35°57'26"N 14°23'27"E	*M. macrostoma*	MN395320, MN395321; MN395351, MN395353
M. m. macrostoma 2	St. Paul's Bay, Mistra, Malta	MR	35°57'18"N 14°23'08"N	*M. macrostoma*	MN395322, MN395323; MN395352, MN395354
M. m. mamotica	Il-Fekruna promontory, Munxar, Gozo	MAM	36°01'59"N 14°13'48"E	*M. mamotica*	MN395326, MN395328; MN395357, MN395358
M. m. oscitans	Mdina, Malta	MW2	35°53'09"N 14°24'03"E	*M. mamotica*	MN395329; MN395361
M. m. macrostoma x *oscitans*	Baharija, Rabat, Malta	MAS	35°53'47"N 14°20'50"E	*M. oscitans*	MN395334, MN395332; MN395364, MN395365
M. m. macrostoma 3	Wied il-Kbir, Qormi, Malta	QC	35°51'56"N 14°28'21"E	*M. oscitans*	MN395336, MN395337 MN395367, MN395368
M. m. oscitans	Ħaġar Qim, Qrendi, Malta	HQ	35°49'36"N 14°26'30"E	*M. oscitans*	MN395331, MN395335; MN395363, MN395362
M. m. oscitans	Mdina, Malta	MW1	35°53'09"N 14°24'03"E	*M. oscitans*	MN395333; MN395366
M. m. macrostoma x *oscitans*	Fort St. Elmo, Valletta, Malta	MAO	35°54'07"N 14°31'04"E	*M. oscitans*	MN395330, MN395327; MN395359, MN395360
M. m. scalaris	St. Paul's Bay, Mistra, Malta	MS	35°57'35"N 14°23'41"E	*M. m. scalaris*	MN395324, MN395325; MN395355, MN395356
Lampedusa (*imitatrix*) *imitatrix*	Fomm Ir-Rih, Rabat, Malta	LIM	35°54'11"N 14°20'03"E	*Lampedusa* (*imitatrix*) *imitatrix*	MN395310, MN395313; MN395344, MN395345
Lampedusa (*imitatrix*) *imitatrix*	Fort St. Elmo, Valletta, Malta	LIMS	35°54'07"N 14°31'04"E	*Lampedusa* (*imitatrix*) *imitatrix*	MN395311, MN395312; MN395346, MN395347
M. syracusana	Roman Amphitheatre Siracusa, Italy	SYR	37°04'28"N 15°16'45"E	*M. syracusana*	HQ696868; HQ696869
M. neuteboomi	Cava di Ispica, Ragusa, Italy	NEU	36°51'11"N 14°50'14"E	*M. neuteboomi*	HQ696866; HQ696867
M. brancatoi	Spinagallo, Siracusa, Italy	SPI	37°00'12"N 15°10'50"E	*M. brancatoi*	MN395314, MN395315, MN395316; KC550118, KC550119, KC550120
M. cyclopica	Epipoli, Castello Eurialo, Siracusa, Italy	EPI	37°05'20"N 15°13'49"E	*M. cyclopica*	MN395317, MN395318, MN395319; MN395348, MN395349, MN395350

Table 1. Data on specimens employed for the present study including initial taxonomic classification, sampling localities, voucher labels, coordinates, final revised taxonomic classification and GenBank Accession Numbers.

1774) (JF277389; JF802033) were used as Out-Groups (OGs).

Phylogenetic analyses

All sequences were visualized with BioEdit Sequence Alignment Editor 7 (Hall, 1999), aligned with the ClustalW option included in this software and refined by eye. Genetic distances were assessed as p distances. Gene sequences were analysed by either single or combined analysis. Phylogenetic analyses were conducted in BEAST 1.6.1 (Drummond & Rambaut, 2007) using the *BEAST implementation (Heled & Drummond, 2010). A series of initial runs was performed to optimize priors and runtime parameter choices to obtain effective sampling sizes (ESS) above 500 for all estimated parameters. Parameter estimates were gained from combined log files. The best-fit evolution model of nucleotide substitution resulted in HKY+G for both genes with empirical base composition; the Yule Process tree prior for mitochondrial data with piecewise linear population size model was applied with a UPGMA-generated tree as the starting point. Five

single runs were combined with the LogCombiner 1.6.1 implemented in the software package BEAST. Trees from all runs were combined to produce an ultrametric consensus tree using TreeAnnotator 1.6.1. The first 10^5 trees were discarded as burnin. Support for nodes is expressed as posterior probabilities.

RESULTS

Partial sequences of the 16S rDNA and COI molecular markers of all the sampled specimens of *Muticaria* from Maltese Islands were analyzed along with the homologous sequences of the four Sicilian species of *Muticaria* in addition to those of two populations of *Lampedusa imitatrix*.

The cladogram in figure 1, as a result of 16S rDNA sequences analysis, shows three mega-clusters: *Lampedusa imitatrix* (in green), three clusters of the *Maltese Muticaria* taxa (in red), including *M. macrostoma+scalaris*, *M. oscitans* and *M. mamotica*, and four clusters (in blue) of the Sicilian *Muticaria* species.

The obtained phylogenetic tree of the Maltese and Sicilian taxa are clearly distinct, and the high posterior probability values are a confirmation of the reliability of this reconstruction.

As a result of COI sequences analysis, the clusters are exactly the same as those already described, and the output does not change (Fig. 2).

After Combined analyis (Fig. 3) as well, clusters result exactly the same as those described before.

Finally, to add further information on the genetic distance between the examined taxa, genetic distances have also been calculated. In particular, for both 16S rDNA and COI partial sequences, p distances between all the sampled populations (Tables

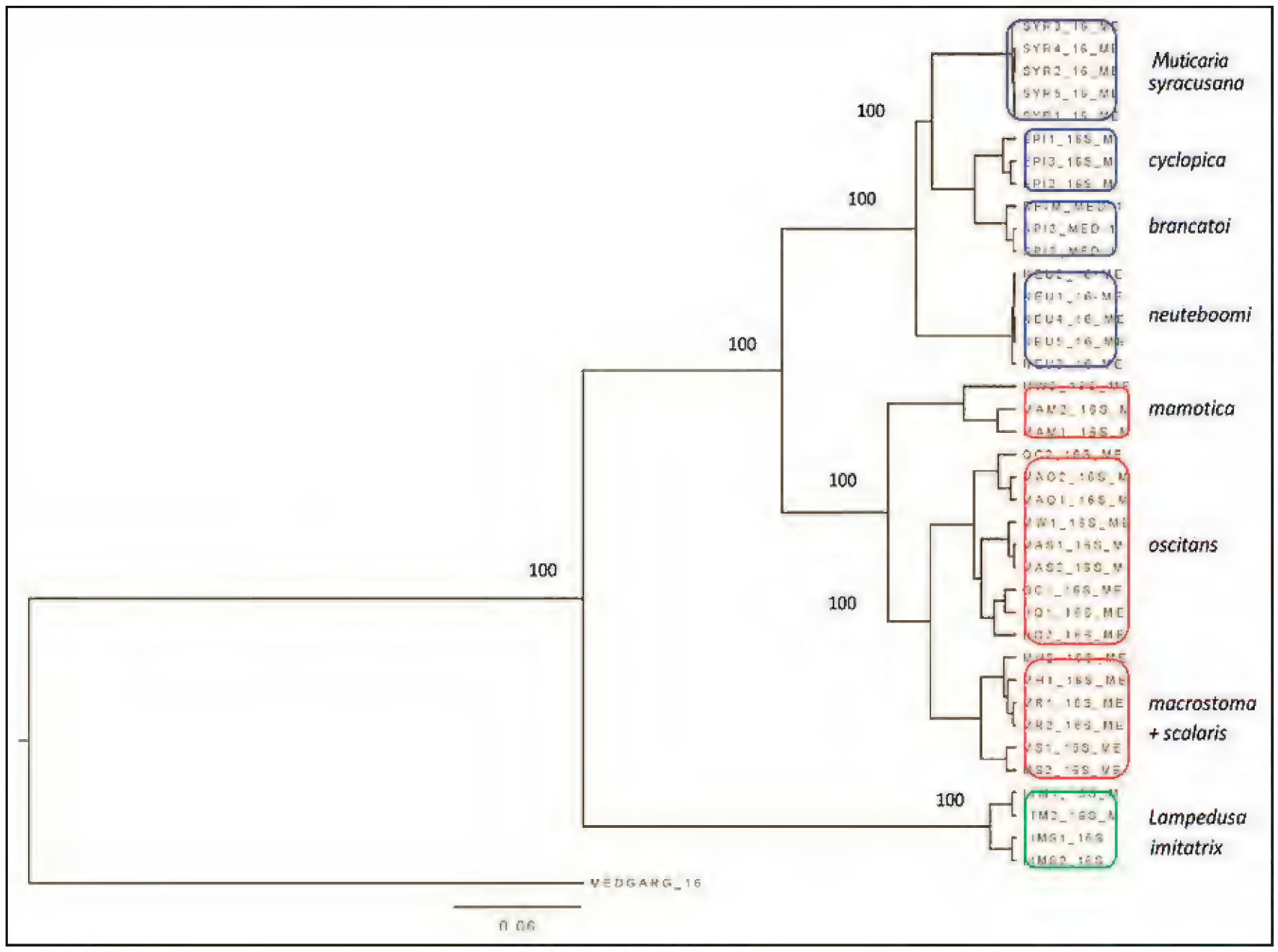

Figure 1. Phylogenetic reconstruction by Bayesian Inference obtained by single gene analysis of 16S rDNA partial sequences. Posterior Probabilities are reported on nodes. Maltese *Muticaria* taxa are shown in red clusters, Sicilian *Muticaria* taxa are shown in blue clusters and *Lampedusa imitatrix* specimens in green clusters.

2, 4) and between the populations grouped by species (Tables 3, 5) were assessed.

DISCUSSION

The results obtained from this work, continuing the studies conducted on the *Muticaria* genus (Colomba et al., 2010, 2012, 2017; Liberto et al., 2016), suggest that the taxa hitherto considered as subspecies of *M. macrostoma* may (with the exception of "*scalaris*") be elevated to the specific rank, on the basis of both the phylogenetic trees topography and genetic p distance values.

In particular, if we consider 16S rDNA p distances >0.2 as delimiting taxa at the genus level and from 0.1 to 0.05 as delimiting taxa at the species rank, it is possible to maintain that, as far as concerns the Maltese taxa:

(i) *M. mamotica* appears very different from *M. macrostoma* (ca. p= 0.103) and *M. oscitans* (p= 0.092);

(ii) *M. oscitans* can be elevated to the species rank with respect to *M. macrostoma* (p= 0.072) and *M. mamotica* (p= 0.092), whereas

(iii) "*scalaris*" seems to fall into *M. macrostoma* (p= 0.032) leaving at the moment unsolved the question of whether to consider it a subspecies or a synonym.

the result of the molecular data for the populations marked with the voucher labels MAO and MAS (apparently hybrid populations, *M. macrostoma* x *oscitans*, see Table 1) is significant of the difficulties that occur in the study of this group by a morphological and anatomical approach only. The shape and the ribs of the shell of these two populations did not allow a sure taxonomic classification. Molecular data indicated that they

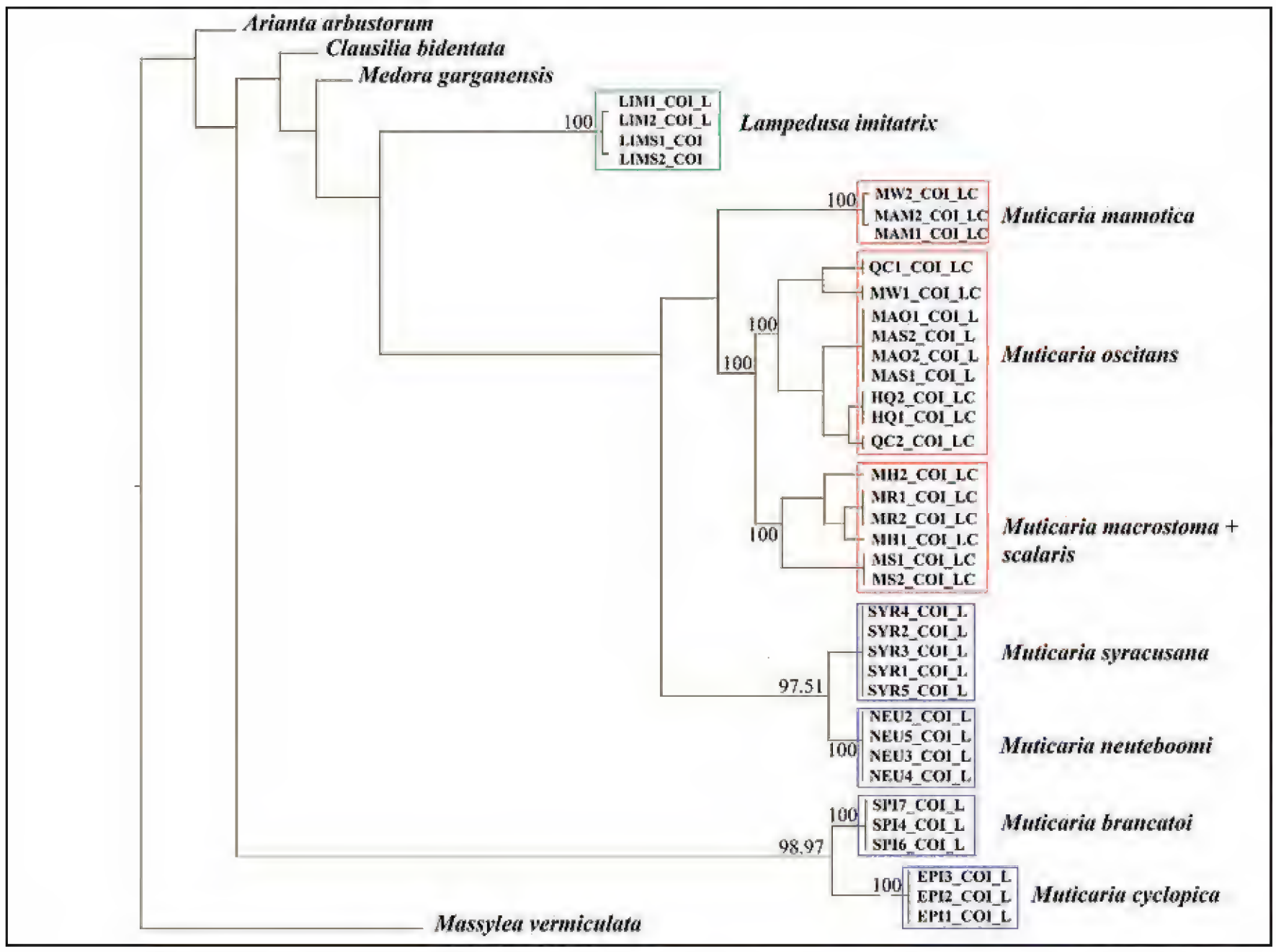

Figure 2. Phylogenetic reconstruction by Bayesian Inference obtained by single gene analysis of COI partial sequences. Maltese *Muticaria* taxa are shown in red clusters, Sicilian *Muticaria* taxa are shown in blue clusters and *Lampedusa imitatrix* specimens in green clusters.

were two populations attributable to *M. oscitans* clade. The molecular diversity of *M. oscitans* (see also Fiorentino et al., 2017) requires further studies.

Sicilian *Muticaria* species are confirmed with *M. syracusana* being clearly distinct from *M. neuteboomi* (p= 0.066) and a little less from *M. brancatoi* (p= 0.046); interestingly, 16 S rDNA p distance between *M. brancatoi* and *M. cyclopica* is quite low (p= 0.021). This is most likely because the two taxa are very similar in the (short) part of the 16S rDNA sequence analyzed, but the topography of the cladogram shows the taxa clearly distinct.

A very similar picture emerges from the analysis of COI p distances. Briefly, from COI amplicons the validity of the Maltese species *M. macrostoma*, *M. mamotica* and *M. oscitans* is confirmed. For the Sicilian species, in addition to *M. syracusana* and *M. neuteboomi*, COI sequences also reveled a significant distance between *M. brancatoi* and *M. cyclopica* (0.626). These molecular data with the known morphological and anatomical differences (Colomba et al., 2012; Liberto et al., 2016) confirm that two different species can be considered.

It should also be noted on all the cladograms examined, how the Sicilian *Muticaria* populations remain distinct from the Maltese ones, clearly indicating two geographical differentiation levels.

Based on the 16S rDNA distances, *Lampedusa* is confirmed as a different genus (p= 0.206) from *Muticaria*. COI distances confirm that they are different genera (*Lampedusa imitatrix* - *Muticaria macrostoma* group p distance= 0.139; *L. imitatrix* - Sicilian *Muticaria* species p distance = 0.261) considering also the known morphological and anatomical differences (Giusti et al., 1995).

The molecular and morphological data obtained

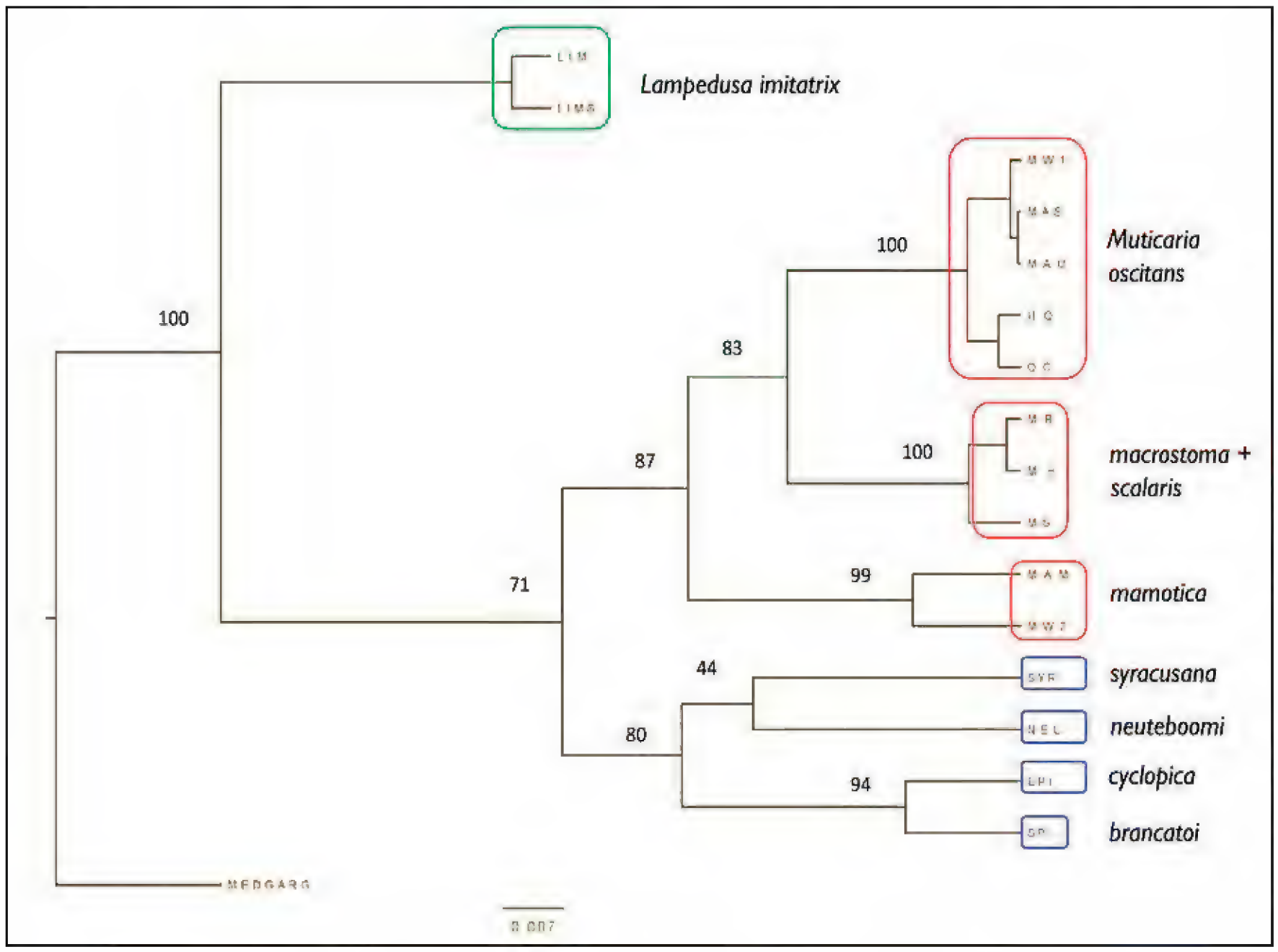

Figure 3. Phylogenetic reconstruction by Bayesian Inference obtained by combined gene analysis of 16S and COI rDNA partial sequences (i.e. concatenated analysis). Posterior Probabilities are reported on nodes. Maltese *Muticaria* taxa are shown in red clusters, Sicilian *Muticaria* taxa are shown in blue clusters and *Lampedusa imitatrix* specimens in green clusters.

	EPI	HQ	LIM	LIMS	MAM	MAO	MAS	MED	MH	MR	MS	MW1	MW2	NEU	QC	SPI	SYR
EPI																	
HQ	0.121																
LIM	0.202	0.171															
LIMS	0.200	0.162	0.018														
MAM	0.117	0.091	0.157	0.155													
MAO	0.130	0.023	0.175	0.165	0.096												
MAS	0.135	0.031	0.166	0.157	0.095	0.037											
MED	0.291	0.277	0.274	0.262	0.288	0.290	0.277										
MH	0.137	0.065	0.175	0.166	0.109	0.070	0.072	0.281									
MR	0.135	0.067	0.174	0.164	0.113	0.072	0.074	0.286	0.009								
MS	0.121	0.061	0.177	0.168	0.105	0.058	0.070	0.290	0.033	0.032							
MW1	0.131	0.028	0.169	0.160	0.091	0.033	0.003	0.281	0.068	0.070	0.067						
MW2	0.115	0.082	0.182	0.172	0.045	0.091	0.093	0.286	0.087	0.089	0.089	0.089					
NEU	0.054	0.108	0.204	0.196	0.120	0.117	0.122	0.279	0.139	0.137	0.123	0.119	0.117				
QC	0.119	0.021	0.173	0.164	0.093	0.019	0.035	0.281	0.077	0.079	0.068	0.031	0.085	0.108			
SPI	0.021	0.123	0.205	0.197	0.127	0.133	0.136	0.278	0.135	0.133	0.132	0.132	0.118	0.058	0.123		
SYR	0.044	0.112	0.198	0.188	0.120	0.124	0.122	0.289	0.132	0.130	0.123	0.119	0.117	0.066	0.114	0.046	

Table 2. p distances between all the sampled populations calculated on 16S rDNA partial sequences (acronyms in Table 1).

	M. cyclopica	M. oscitans	L. imitatrix	M. mamot.	Med. garg.	M. macrost	scalaris	M. neuteb.	M. brancat.	M. syrac.
M.cyclopica										
M. oscitans	0.127									
L. imitatrix	0.201	0.166								
M. mamot.	0.116	0.092	0.163							
Med. garg.	0.291	0.281	0.268	0.287						
M. macrost.	0.136	0.072	0.170	0.103	0.284					
scalaris	0.121	0.065	0.173	0.100	0.290	0.032				
M. neuteb.	0.054	0.115	0.200	0.119	0.279	0.138	0.123			
M. brancat.	0.021	0.129	0.201	0.124	0.278	0.134	0.132	0.058		
M. syrac.	0.044	0.118	0.193	0.119	0.289	0.131	0.123	0.066	0.046	

Table 3. 16S rDNA p distances between all the sampled populations arranged by species.

	EPI	HQ	LIM	LIMS	MAM	MAO	MAS	MED	MH	MR	MS	MW1	MW2	NEU	QC	SPI	SYR
EPI																	
HQ	0.591																
LIM	0.605	0.128															
LIMS	0.609	0.135	0.021														
MAM	0.592	0.088	0.160	0.147													
MAO	0.601	0.033	0.132	0.133	0.100												
MAS	0.600	0.033	0.132	0.134	0.094	0.000											
MED	0.614	0.174	0.187	0.195	0.185	0.181	0.180										
MH	0.595	0.080	0.143	0.150	0.092	0.075	0.074	0.172									
MR	0.595	0.078	0.139	0.146	0.094	0.073	0.072	0.169	0.010								
MS	0.589	0.075	0.141	0.146	0.092	0.070	0.069	0.177	0.017	0.022							
MW1	0.593	0.029	0.134	0.137	0.091	0.003	0.003	0.179	0.071	0.068	0.065						
MW2	0.586	0.084	0.150	0.148	0.051	0.100	0.100	0.177	0.094	0.098	0.095	0.098					
NEU	0.610	0.117	0.160	0.157	0.141	0.125	0.126	0.183	0.124	0.125	0.126	0.122	0.131				
QC	0.598	0.036	0.125	0.123	0.090	0.036	0.036	0.158	0.076	0.072	0.074	0.033	0.088	0.107			
SPI	0.626	0.256	0.265	0.285	0.274	0.254	0.259	0.241	0.260	0.257	0.256	0.257	0.277	0.252	0.242		
SYR	0.614	0.128	0.150	0.149	0.134	0.134	0.134	0.191	0.122	0.120	0.130	0.131	0.134	0.121	0.124	0.254	

Table 4. p distances between all the sampled populations calculated on COI partial sequences (acronyms in Table 1).

	M.cyclopica	M. oscitans	L. imitatrix	M. mamot.	Med. garg.	M. macrost	scalaris	M. neuteb.	M. brancat.	M. syrac.
M.cyclopica										
M. oscitans	0.597									
L. imitatrix	0.607	0.131								
M. mamot.	0.590	0.093	0.152							
Med. garg.	0.614	0.174	0.191	0.182						
M. macrost.	0.595	0.074	0.145	0.094	0.171					
scalaris	0.589	0.071	0.143	0.093	0.177	0.019				
M. neuteb.	0.610	0.119	0.158	0.138	0.183	0.125	0.126			
M. brancat.	0.626	0.253	0.275	0.275	0.241	0.259	0.256	0.252		
M. syrac.	0.614	0.130	0.150	0.134	0.191	0.121	0.130	0.121	0.254	

Table 5. COI p distances between all the sampled populations arranged by species.

by Fiorentino et al. (2017, bioRxiv unpublished preprint) show *Lampedusa* and *Muticaria* as two different genera, and *Muticaria* as a monophyletic clade divided into three geographical lineages (Sicilian, Maltese and Gozitan populations).

CONCLUSIONS

All the above data allow us to draw taxonomic conclusions, even partial, on the *Muticaria* populations examined for their greater knowledge and protection.

In conclusions, our findings suggest that:

(i) within Maltese *Muticaria* is possible to elevate to the specific rank "*mamotica*" and "*oscitans*", thus bringing to three the Maltese *Muticaria* species i.e. *M. macrostoma*, *M. mamotica* and *M. oscitans*; whereas *M. macrostoma scalaris* would remain a subspecies;

(ii) as far as concerns the Sicilian species, *M. syracusana*, *M. neuteboomi*, *M. brancatoi* and *M. cyclopica* are confirmed;

(iii) the Sicilian and Maltese *Muticaria* populations seem to belong to two geographical differentiation levels;

(iv) *Lampedusa* and *Muticaria* are two different genera.

The high level of differentiation found within this group is a consequence both of the complex biogeographical history of this region and of strict connection between the geological (calcareous) nature of the soil these molluscs live in and the scarce vagility of the specimens, leading to island-like distributional patterns characterized by high levels of endemism.

All this requires a greater commitment in the protection and management of these land molluscs and the environments in which they live.

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New data on the genus *Albinaria* Vest, 1867 (Pulmonata Clausiliidae) from the Astypalea Island and neighboring islets (Dodecanese Archipelago, Greece)

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ABSTRACT

In this work, the authors investigated the genus *Albinaria* Vest, 1867 (Pulmonata Clausiliidae) from the Astypalea Island and the nearby islets of Ofidoussa and Kounoupi (Dodecanese Archipelago, Greece). The two endemic subspecies known, *Albinaria* (*Albinaria*) *brevicollis astropalia* (O. Boettger, 1883) and *A.* (*A.*) *brevicollis maltezana* Nordsieck, 2015 are redescribed and illustrated for shell and genital morphology. Furthermore *A.* (*A.*) *brevicollis* cf. *sica* Fuchs et Käufel, 1936 is reported for the first time from the north-east Astypalea, and two new subspecies, *A.* (*A.*) *brevicollis granoi* n. ssp. and *A.* (*A.*) *brevicollis cristinae* n. ssp. are here described from North-West Astypalea and Ofidoussa Islet, respectively.

KEY WORDS Taxonomy; morphology; new subspecies; distribution.

Received 26.07.2018; accepted 03.10.2018; published online 28.12.2019
Proceedings of the 4th International Congress on Biodiversity "Man, Natural Habitats and Euro-Mediterranean Biodiversity", November 17th-19th, 2017 - Malta

INTRODUCTION

The highly diverse genus *Albinaria* Vest, 1867 (Pulmonata Clausiliidae) is present in the Dodecanese Archipelago (South-East Aegean region) with 12 species and 34 subspecies (Bank, 2017, 2019). The species identification is almost exclusively based on shell morphology and recent molecular studies have mainly confirmed species classification based on shell morphology (Douris et al., 2007).

In the Dodecanese Archipelago, *A.* (*Albinaria*) *brevicollis* (L. Pfeiffer, 1850) is the most diversified species with 17 subspecies.

In the Astypalea Island, two endemic subspecies of *A.* (*A.*) *brevicollis* are known: *Albinaria brevicollis astropalia* (O. Boettger, 1883) from the western part of the island (Kora and Livadhi nearby) and *A. brevicollis maltezana* (Nordsieck, 2015) known only from the type locality, mountain ridge North-East Maltezana = Analipsi (Fuchs & Käufel, 1936; K.L. Pfeiffer, 1955; Nordsieck, 2015).

In 2015, Mauro Grano and Cristina Cattaneo (Rome, Italy), during a naturalistic trip, have sampled fifteen populations of *Albinaria* on the Astypalea Island and on the nearby islets of Ofidoussa and Kounoupi.

The examination of this material revealed the presence of five different populations of *A.* (*A.*) *brevicollis*; two correspond to the known endemic subspecies *A.* (*A.*) *brevicollis astropalia* and *A.* (*A.*) *brevicollis maltezana* respectively, the other three

are new to the island group of Astypalea and are discussed below.

MATERIAL AND METHODS

Study area

The island group of Astypalea (Dodecanese Archipelago, Greece) lies in the South-East Aegean Sea, representing a transition zone between the Kiklades islands and the eastern Aegean (Fig. 1). Astypalea is the largest island of the group, with an area of 96 km². It consists of two parts: a western half (Exo Nisi) and an eastern one (Mesa Nisi), joined by a narrow isthmus (Steno), 105 m wide, derived from the collapse of the neighboring territories. The two extreme parts of the island consist mainly of limestone, while the central part is flysch and alluvial (Fig. 2). The highest relief is Vardhia (482 m). Maquis and phrygana constitute the dominant vegetation types with different endemic or rare animal and plant taxa. Astypalea is surrounded by numerous smaller uninhabited off-shore islets, the largest of which are Kounoupi to the southeast and Ofidoussa to the west. Both consist mainly of limestone (Fig. 3).

Sampling methods

All samples examined for this paper were collected by M. Grano and C. Cattaneo, from 2nd August to 18th August 2015 and from 24th April 2016 to 26th April 2016. The names of local places mentioned in the text and in the map (Fig. 1) follow the map of Astypalea produced by Terrain Cartography Group (2009). Specimens were collected chronologically from the following localities:

Astypalea, Livadhi, 36°32'58''N – 26°19'59''E, 51 m, 02.VIII.2015
Astypalea, Vardhia, 36°31'29''N – 26°19'11''E, 375 m, 03.VIII.2015
Astypalea, Vatses, 36°30'53''N – 26°19'13''E, 80 m, 04.VIII.2015
Astypalea, Aghios Konstantinos, 36°31'39''N – 26°21'15''E, 10 m, 06.VIII.2015
Astypalea, Kaminakia, 36°31'19''N – 26°18'14''E, 42 m, 07.VIII.2015
Kounoupi Islet, 36°32'07''N – 26°28'04''E, 50 m, 10-11.VIII.2015
Astypalea, Dhracospilia 36°38'21''N – 26°22'50''E, 54 m, 13.VIII.2015
Astypalea, Panormos, 36°35'10''N – 26°16'37''E, 16 m, 14.VIII.2015
Astypalea, Pachia Ammos, 36°35'31''N – 26°17'30''E, 41 m, 15.VIII.2015
Ofidoussa Islet, 36°33'12''N – 26°08'23''E, 82 m, 18.VIII.2015
Astypalea, Ftera, 36°32'10''N – 26°18'36''E, 337 m, 18.VIII.2015
Astypalea, Ftera, 36°33'21'"N – 26°17'12'"E, 326 m, 24.IV.2016
Astypalea, Vatses, 36°30'58'"N – 26°19'25'"E, 186 m, 25.IV.2016
Astypalea, Koutela, 36°31'38'"N – 26°18'57"E, 393 m, 26.IV.2016
Astypalea, Kora, 36°32'36"N – 26°20'49"E, 43 m, 26.IV.2016

The land snails were collected by hand on the soil and on the rocks. Dry shells have been studied as regards size, sculpture, aperture, plicae and lamellae, lunella and clausilium. In order to study and illustrate genital organs, the specimens were drowned in water and fixed in 75% ethanol. Reproductive apparatus was extracted by means of scalpel, scissors and needles. Height and maximum diameter of the shell along with some parts of genitalia were measured (in millimeters) with a digital gauge. Photographs were taken with a digital camera. Taxonomical references are based on the checklist of the land and freshwater Gastropoda of Europe (Bank, 2017, 2019). The voucher specimens are deposited in the following Museums and private collections: CG (M. Grano collection, Roma, Italy), CL (F. Liberto collection, Cefalù, Italy), MCZR (Museo Civico di Zoologia, Roma, Italy), CP (G. Pocaterra collection, San Pietro in Casale, Italy), CS (I. Sparacio collection, Palermo, Italy).

ABBREVIATIONS AND ACRONYMS. D: shell diameter, H: shell height, ex: specimen, exx: specimens, R2: ribs number on 2 mm of the penultimate whorl, sh/s: shell/s.

ANATOMICAL ACRONYMS. AG: albumen gland, BC: bursa copulatrix, BCD: diverticulum of bursa copulatrix, CD: copulatory duct, DBC: duct of bursa copulatrix, E: epiphallus, FO: free oviduct, GA: genital atrium, HD: hermaphrodite duct, O: ovotestis, OV: ovispermiduct, P: penis, PC: penial

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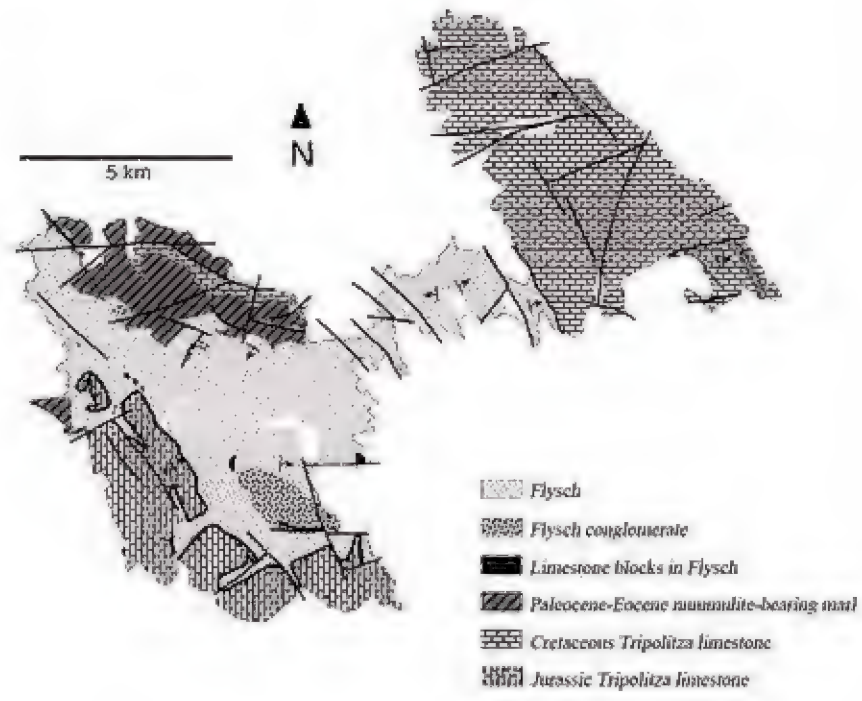

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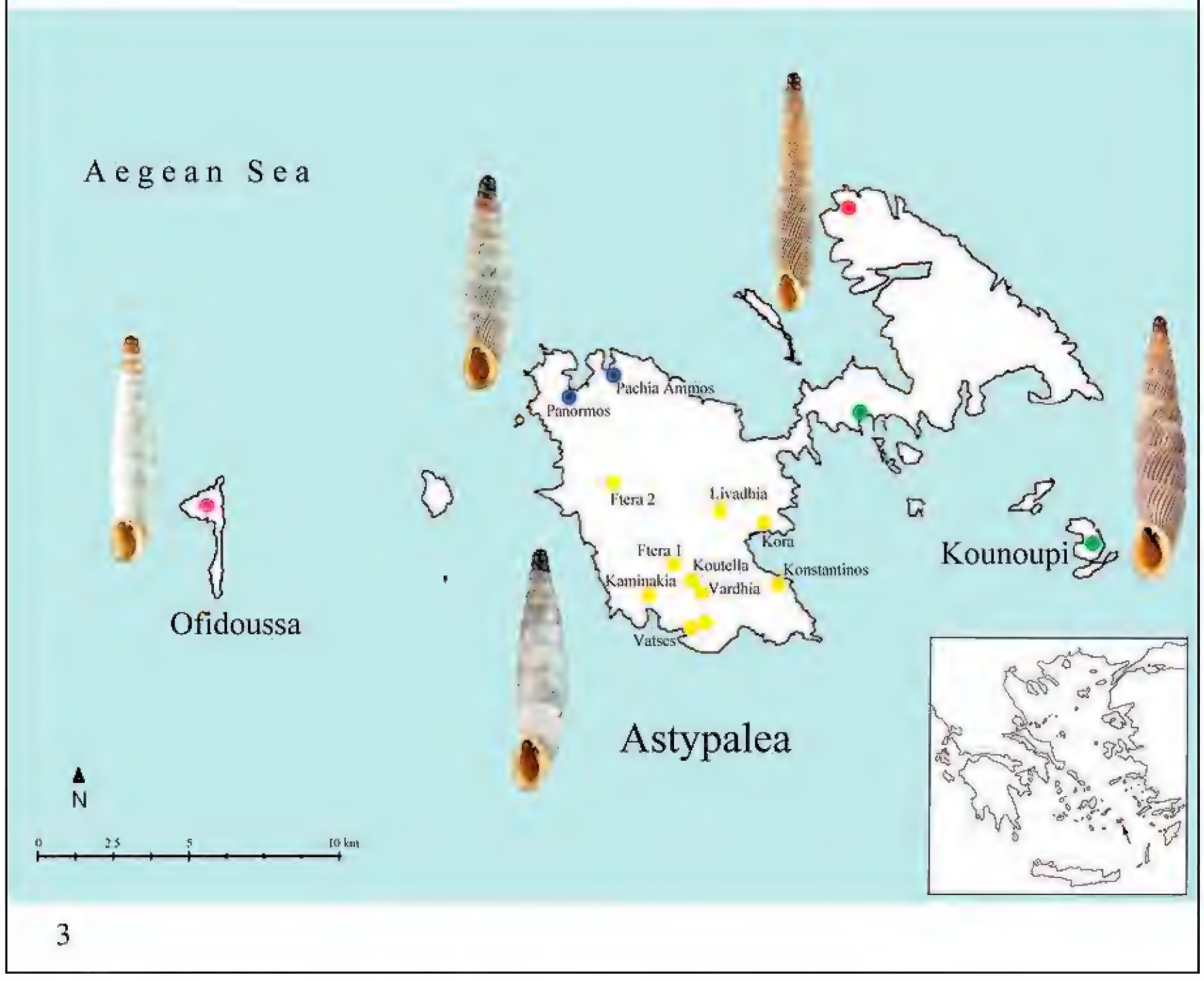

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Figure 1. Map of Dodecanese Archipelago. Figure 2. Geological map of Astypalea Island (modified from Ring, 2001). Figure 3. Distribution map of the subspecies of *Albinaria brevicollis* in the island group of Astypalea; yellow dots: *A. b. astropalia*, green dots: *A. b. maltezana*, red dot: *A. b.* cf. *sica*, blue dots: *A. b. granoi* n. ssp, purple dot: *A. b. cristinae* n. ssp.

caecum, PR: penial retractor muscle, V: vagina, VD: vas deferens, VP: V-shaped pleat.

CONCHOLOGICAL ACRONYMS. B: basal plica (= lower palatal plica), IL: inferior lamella, L: lunella, PP: principal plica, PUPP: posterior upper palatal plica, UL: upper lamella, SCL: subcolumellar lamella, SL: spiral lamella, SS: subclaustralis.

RESULTS

Systematics

Classis GASTROPODA Cuvier, 1795
Ordo STYLOMMATOPHORA Schmidt, 1855
Familia CLAUSILIIDAE Gray, 1855
Subfamily ALOPIINAE A.J. Wagner, 1913
Tribe MEDORINI H. Nordsieck, 1997
Genus *Albinaria* Vest, 1867
Subgenus *Albinaria* Vest, 1867

Albinaria (Albinaria) brevicollis astropalia

(O. Boettger, 1883)

EXAMINED MATERIAL. Greece, Southwest Astypalea Island, Livadhi, 36°32'58.11'' N 26°19'59.64'' E, 51 m, 02.VIII.2015, 2 exx, 4 shs (CL 158–163; Figs. 14–15 genitalia CL 158); idem, Ftera, 36°32'10.95''N 26°18'36.79''E, 337 m, 18.VIII.2015, 6 exx. (CL 164-169); idem, Vardhia, 36°31'29.48''N 26°19'11.50''E, 375 m, 03.VIII.2015, 5 exx, 3 shs (CL 170–177; Fig. 7 parietum CL 171); idem Vatses, 36°30'53.86''N 26°19'13.46''E, 80 m, 04.VIII.2015, 6 exx, 2 shs, (CL 178–185; Fig. 6 parietum CL 178); idem, Aghios Konstantinos, 36°31'39.53''N 26°21'15.53''E, 10 m, 06.VIII.2015, 5 exx, 4 shs (CL 187-195; Fig. 5 shell CL 195; Fig. 8 palatum CL 187; Fig. 13 genitalia (CL 188.); idem, Kaminakia, 36°31'19.32''N 26°18'14.98''E, 42 m, 07.VIII.2015, 7 exx, (CL 196–202); idem, 2 exx (CS); idem, Kora, 36°32'36"N 26°20'49"E, 43 m, 26.IV.2016, 1 ex, 6 shs, (CL373–379); idem, Ftera, 36°33'21,45"N 26°17'39,12"E, 326 m, 24.IV.2016, 7 exx, 2 shs, (CL 381–389; Fig. 4 shell CL 388; Fig. 10 clausilium CL 382; Figs. 11–12 genitalia CL 381); idem, Vatses, 36°30'58,67"N 26°19'25,63"E, 186 m, 25.IV.2016, 7 exx, (CL 399–405); idem, Koutela, 36°31'38,68"N 26°18'57,62"E, 393 m, 26.IV.2016, 9 exx, 1 sh, (CL 411–424; Fig. 9 palatum CL 420).

DESCRIPTION. Fusiform shell more or less slender, white bluish in color, with smooth whorls, some ribs are present only on the last half of the last whorl, almost absent dorsal keel (Figs. 4, 5); principal plica well developed, posterior upper palatal plica fused with lunella apex; lunella dorsolateral, in part with basalis rudiment (Figs. 6, 7); superior lamella reaching spiral lamella.

Genitalia typical of *A.* (*A.*) *brevicollis* with a well developed cylindrical or conical penial caecum (1–2.6 mm, mean 1.6 mm) a V-shaped crest inside the penis (Figs. 11–15).

Measurements of the shell: H: 13.7–19.7 mm, (mean: 16 mm), D: 3–4.16 mm (mean: 3.6 mm), whorls: 9–11.75 (mean: 10.5).

DISTRIBUTION. The type of *A.* (*A.*) *brevicollis astropalia* comes from Kora (West Astypalea). Nordsieck (2015) ascribes also the population from Livadhi locality. Based on our sampling *A.* (*A.*) *brevicollis astropalia* is also present in Ftera, Vardhia, Koutela, Vatses and Aghios Konstantinos locality (South-West Astypalea).

REMARKS. The populations of Konstantinos, with H: 14.46, D: 3.2 and 10 whorls, is the smallest population of *A.* (*A.*) *brevicollis astropalia.*

Albinaria (Albinaria) brevicollis maltezana

Nordsieck, 2015

EXAMINED MATERIAL. Kounoupi Islet, southeast Astypalea Island, Greece, 36°32'07.48''N 26°28'04.88''E, 50 m, 10–11.VIII.2015, 10 exx, 4 shs (CL 316–331; Figs. 16–17 shells CL 326-327; Figs. 18-19 parietum CL 318–319; Figs. 20–21 palatum CL 330–331; Figs. 22, 23 clausilium Cl 330, 320; Figs. 24–27 genitalia CL 316–317; idem 2 exx (CS).

DESCRIPTION. Shell distinctly ribbed, dorsal keel mostly prominent; superior lamella often reaching or surpassing spiral lamella, means dimensions H: 16.3 mm, D: 3.4 mm, R2 (n = 11): 8–12 (mean 9.9).

Dimension of genitalia (means of two specimens examined): P: 2.6 mm, E: 3.9 mm, PC: 2.25 mm; vagina: 4.6 mm, CD 1.4 mm, DBC+BC 3.3 mm, BCD: 7 mm.

DISTRIBUTION. *Albinaria* (*A.*) *brevicollis maltezana* is known only for the type locality: mountain ridge North East Maltezana = Analipsi (Nordsieck, 2015).

REMARKS. We ascribe to this subspecies the population of the small islet of Kounoupi (8 km South-East of Maltezana), which shows the same mean dimensions and the same distinctive characters in the shell (Figs. 16–23). We observed some specimens with the lower part of the lunella bent inwards = rudiment subclaustralis (Fig. 19). We examined the genitalia of two specimens. They show a very long vagina of 6 mm and 4.35 mm respectively, and diverticulum of the bursa copulatrix of 9 mm and 7 mm, respectively. However, this may be due to a recent mating, as evidenced by the presence of the spermathophora inside the diverticulum (Figs. 24–27). We have also sampled on the small islet of Koutsomiti, but here we have not found *Albinaria.*

Albinaria (Albinaria) brevicollis cf. ***sica*** Fuchs et Käufel, 1936

EXAMINED MATERIAL. Astypalea, Dhracospilia, near the cave Dhracospilia, Northeast Astypalea, Greece, 36°38'21.56''N 26°22'50.25''E, 54 m, 13.VIII.2015, 8 exx, 6 sh (CL 214–224; Figs. 28–29 shells CL 214, 224; Figs. 30–31 parietum CL 215–216; Figs. 32–33 palatum CL 225–226; Fig. 34 clausilium CL 225; Figs. 35–36 genitalia CL 215; Figs. 37–38 genitalia CL 216); idem 2 exx (CS).

DESCRIPTION. Slender conical shell; 1.5–2 apical whorls convex and brown; the other whorls convex, uniformly ribbed, brown in colour with white ribs; irregular ribs on the last part of the last whorl; dorsal keel low or moderately high; peristome oval detached (Figs. 28–29); lower part of lunella with a rudiment basalis or bent inwards = rudiment subclaustralis (Figs. 30–31), upper lamella rarely reaches the spiral lamella (Figs. 32–33, 35–38).

Dimensions of the shell: H: 13.7–16.5 mm, (mean 15.5 mm), D: 2.8–3.3 mm (mean 3.2 mm), whorls: 10–12.5 (mean 11.3), R2 (n = 9): 9–15 (mean 11.5).

Dimensions of the genitalia (two specimens examined): P: 1.5–2.4 mm, E: 2–3.15 mm, PA: 0.7–2 mm; V: 1.5–3.4 mm, CD 1–1.1 mm, DBC+BC 1–2 mm, BCD: 2.9–4.7 mm

REMARKS. The *Albinaria* population of Dhracospilia is very similar to *A.* (*A.*) *brevicollis sica* described for the island of Megali Zafora, the largest and northernmost of the Zafora islands (about 50 km southeast of Astypalea). We attribute the Dhracospilia population to this subspecies, but further sampling and examination are desiderable to clarify the philogenetic relations both with *A.* (*A.*) *brevicollis sica* from the type locality both with *A.* (*A.*) *brevicollis maltezana.*

Albinaria (Albinaria) brevicollis granoi n. ssp.

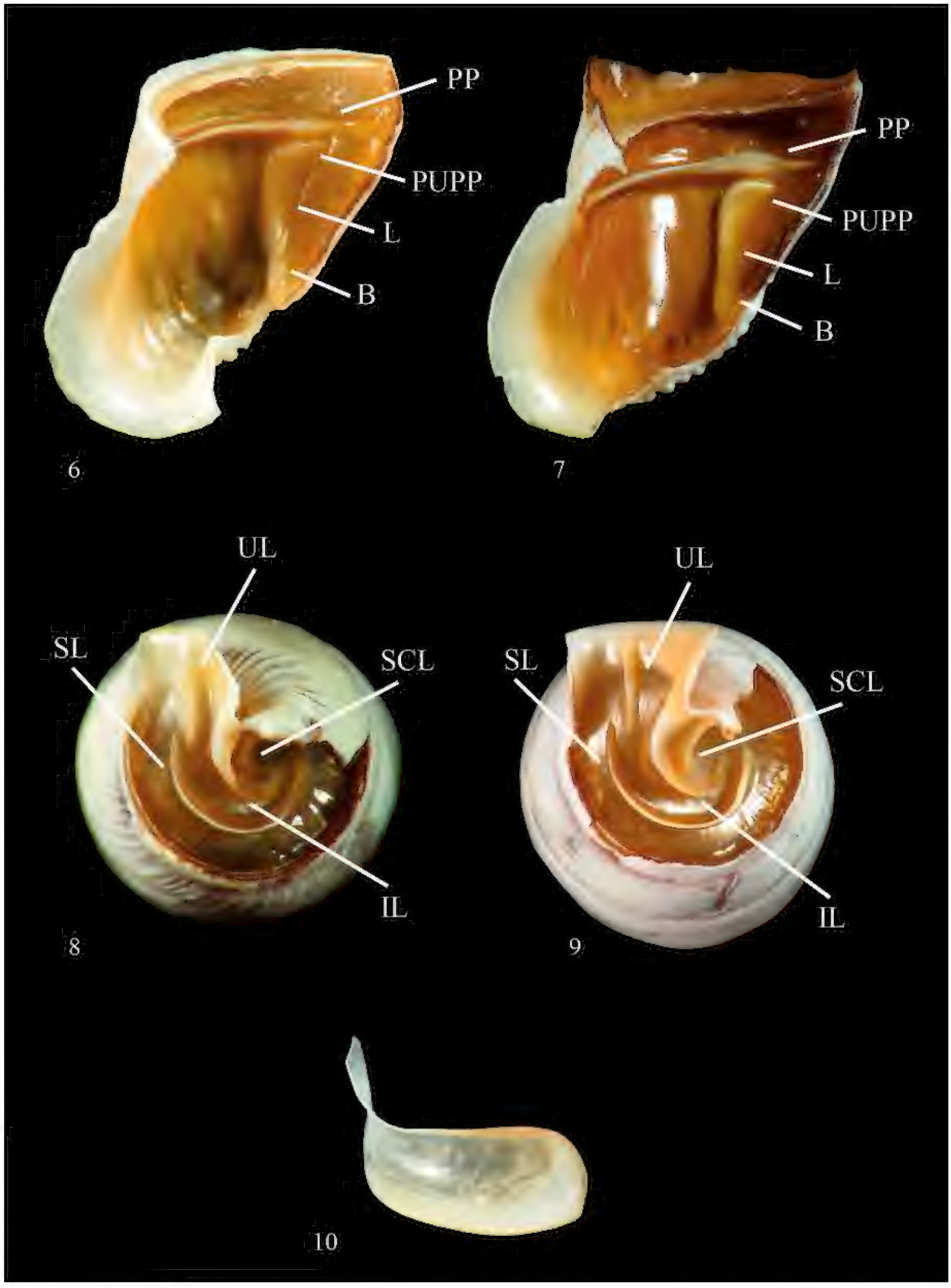
TYPE LOCALITY. Pachia Ammos, North-West Astypalea Island, Dodecanese Archipelago, Greece.

TYPE MATERIAL. Holotype (Fig. 39): Pachia Ammos, on the rocks close to the sea, North-West Astypalea Island, 36°35'31.15''N 26°17'30.12''E, 41 m, M. Grano and C. Cattaneo legit, 15.VIII.2015, (MCZR-M-TYPE 00250/H). Paratypes: idem 8 exx., 11 shs, (CL 240–258; Fig. 40 shells CL 249; Figs. 41–42 parietum CL 241, 251; Figs. 43–44 palatum CL 250, 251; Figs. 45–46 clausilium CL 242, 251; Figs. 47–48 genitalia CL 240; Figs. 49–50 genitalia CL 241); idem 2 shs (CS); idem 2 shs (CG); Panormos, on the rocks close to the sea, North-West Astypalea Island, 36°35'10.26''N 26°16'37.62''E, 16 m, 14.VIII.2015, 1 ex, 5 shs (CL 230–235); idem 2 shs (CP).

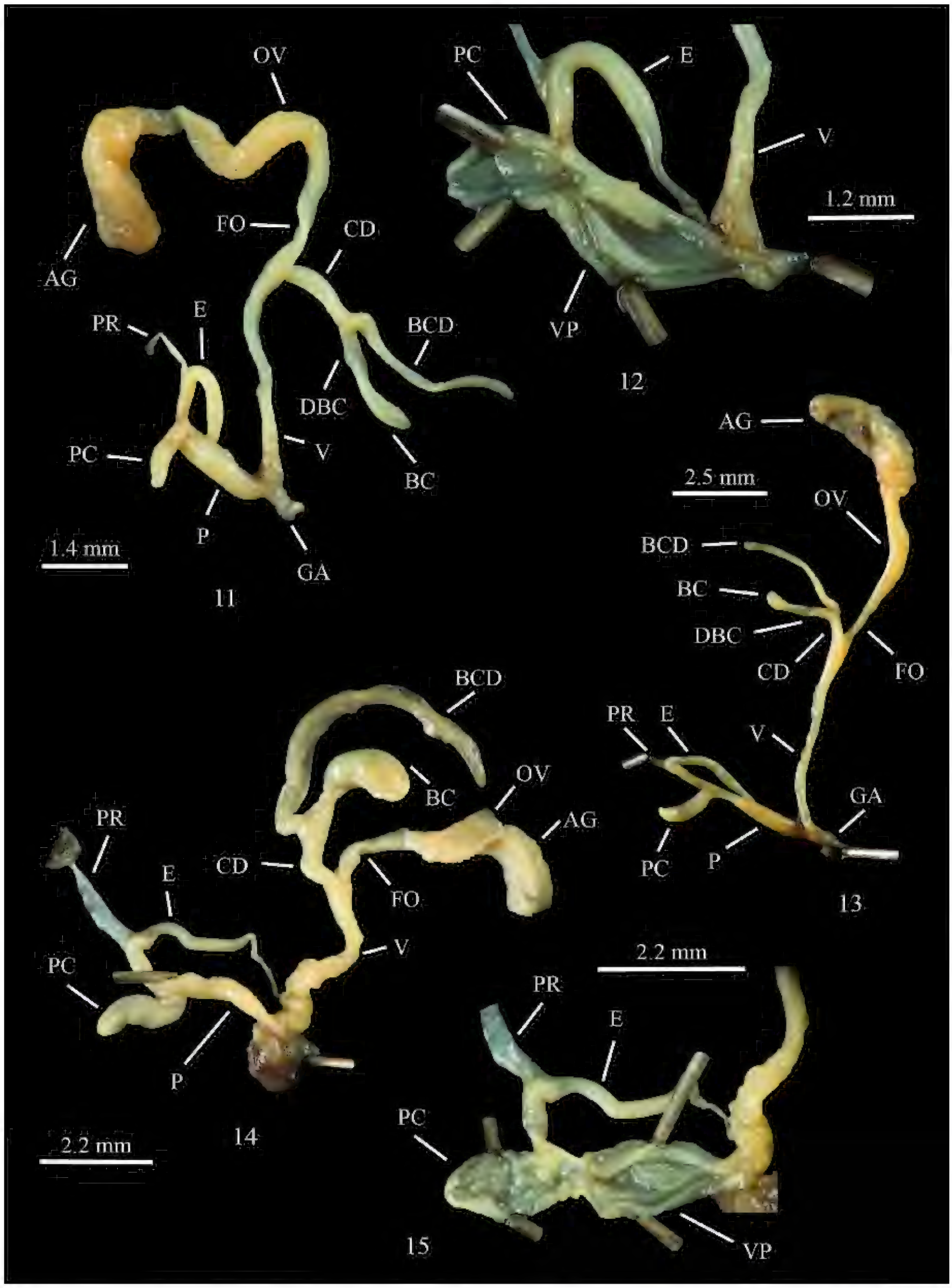
DIAGNOSIS. Spindle-shaped, medium-small shell H: 13.9, D: 2.9, with 10.5 whorls (mean of 15 shells), characterized by rounded apical and subapical whorls, spire ribbed, basal keel and dorsal keel distinct, upper lamella reaches or does not reach spiral lamella.

DESCRIPTION OF THE HOLOTYPE (Fig. 39). Spindle-shaped shell, H: 12.9 mm, D: 2.9 mm; with 10 ½ whorls, the apical and subapical whorls are more convex than the subsequent ones, the two apical whorls are dark brown, the following white with ample brown spots and points and with uniformly white ribs, R2: 14; on the last part of the last whorl the ribs are irregular; sutural bulge present, basal keel distinct, dorsal keel about as high as basal keel and shorter, detached oval peristome; upper lamella reaching the spiral lamella; inferior lamella low within, ending on columellar edge in front; lower part of lunella with a rudiment of basalis.

Figure 4. Shell of *Albinaria* (*Albinaria*) *brevicollis astropalia* Ftera, Astypalea Island, Greece, H: 15.1 mm, D: 3.6 mm.
Figure 5. Idem, Aghios Konstantinos, Astypalea Island, Greece, H: 15.2 mm, D: 3.3 mm.

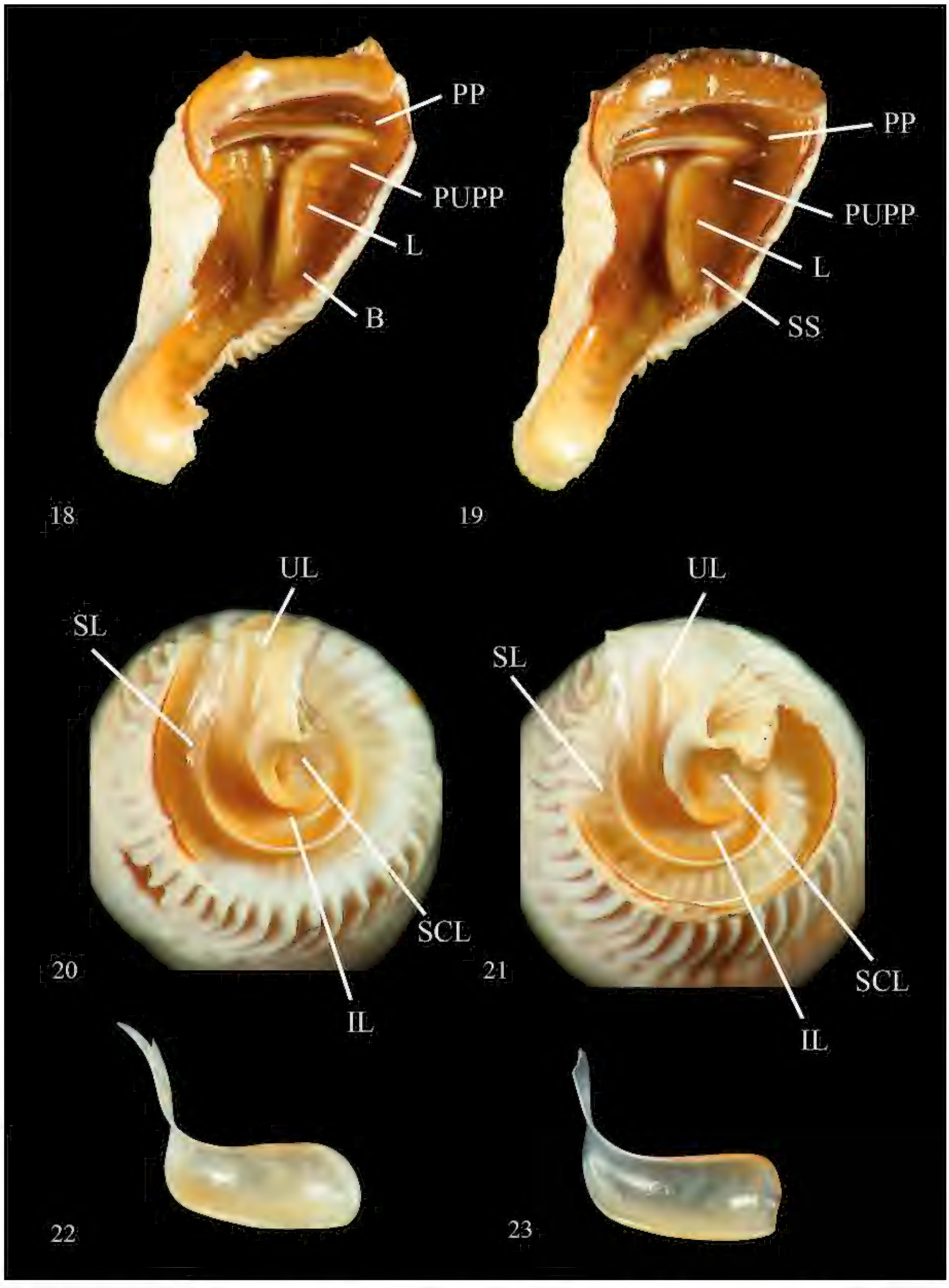


Figures 6–10. Parietum, palatum and clausilum of *Albinaria* (*Albinaria*) *brevicollis astropalia,* Astypalea Island, Greece. Fig. 6. Parietum: Astypalea, Livadhia; Fig. 7. Parietum: Astypalea, Vardhia, Fig. 8. Palatum: Astypalea, Konstantinos; Fig. 9. Palatum: Astypalea, Koutella, Fig. 10. Clausilium: Astypalea, Ftera.

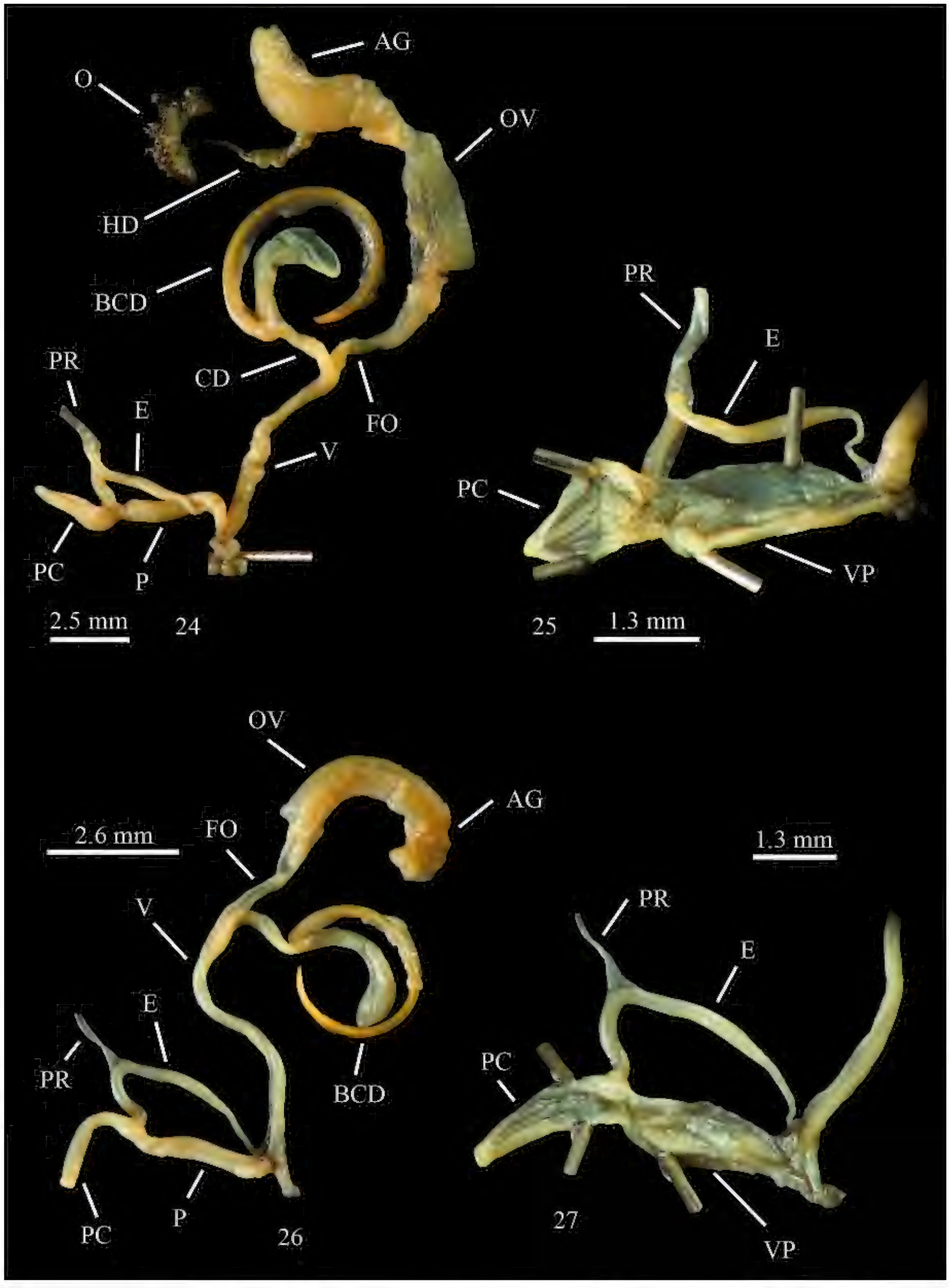


Figures 11–15. Genialia of *Albinaria* (*Albinaria*) *brevicollis astropalia*, Astypalea Island, Greece. Fig. 11. Genitalia: Ftera. Fig. 12. Internal structure of penis, same specimen of figure 11. Fig. 13. Genitalia: Konstantinos. Fig. 14. Genitalia: Livadhia. Fig. 15. Internal structure of penis, same specimen of figure 14.

Fig. 16. Shell of *Albinaria* (*Albinaria*) *brevicollis maltezana*, Kounoupi Islet, islands group of Astypalea, Greece, H: 18.1 mm, D: 3.6 mm. Fig. 17. idem, H: 14.9 mm, D: 3.3 mm.

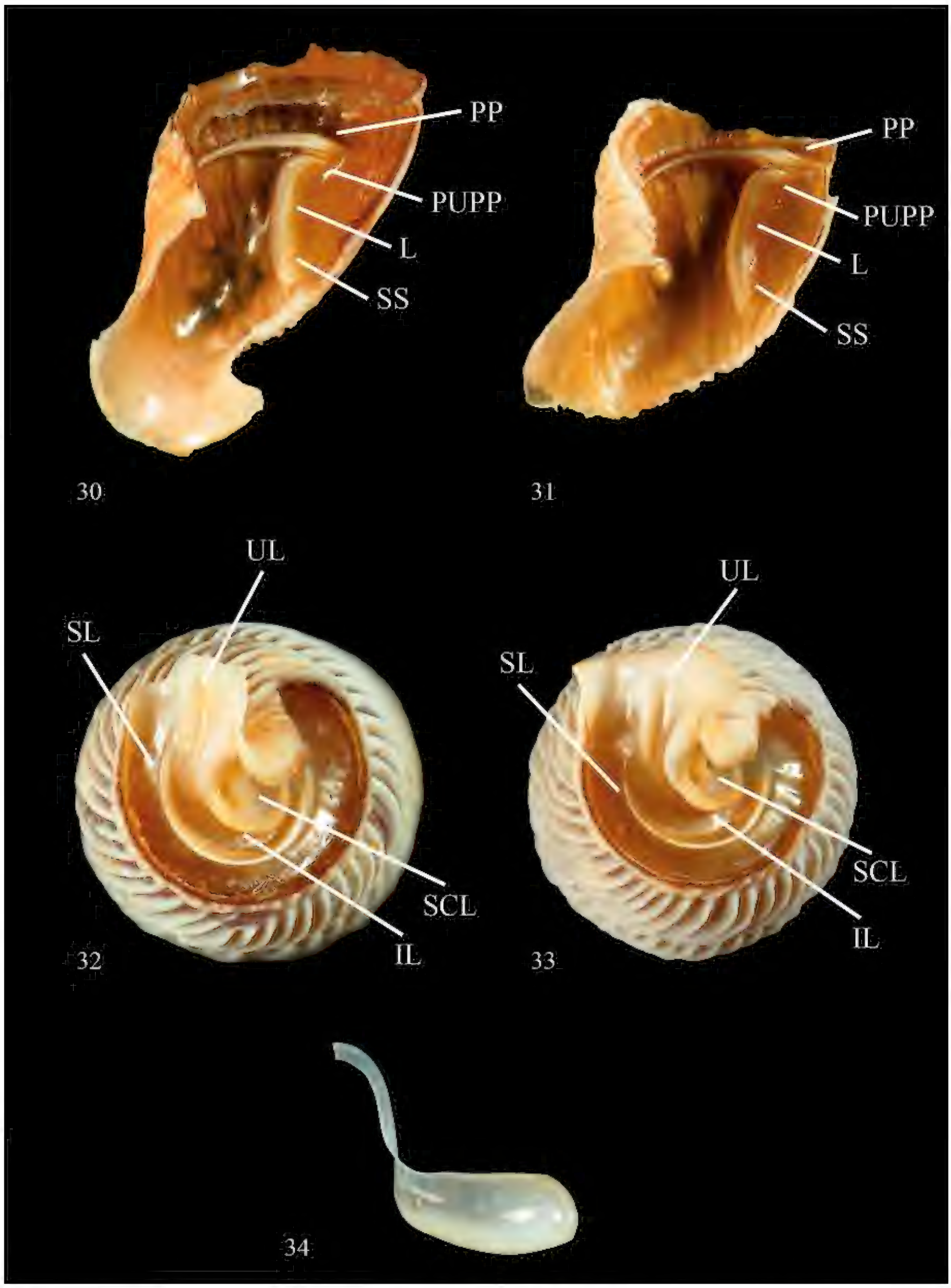


Figures 18–23. Parietum, palatum and clausilium of *Albinaria* (*Albinaria*) *brevicollis maltezana*, Kounoupi Islet, island group of Astypalea, Greece. Fig. 18. Parietum. Fig. 19. Parietum. Fig. 20. Palatum. Fig. 21. Palatum. Fig. 22. Clausilium, same specimen of figure 20. Fig. 23. Clausilium.

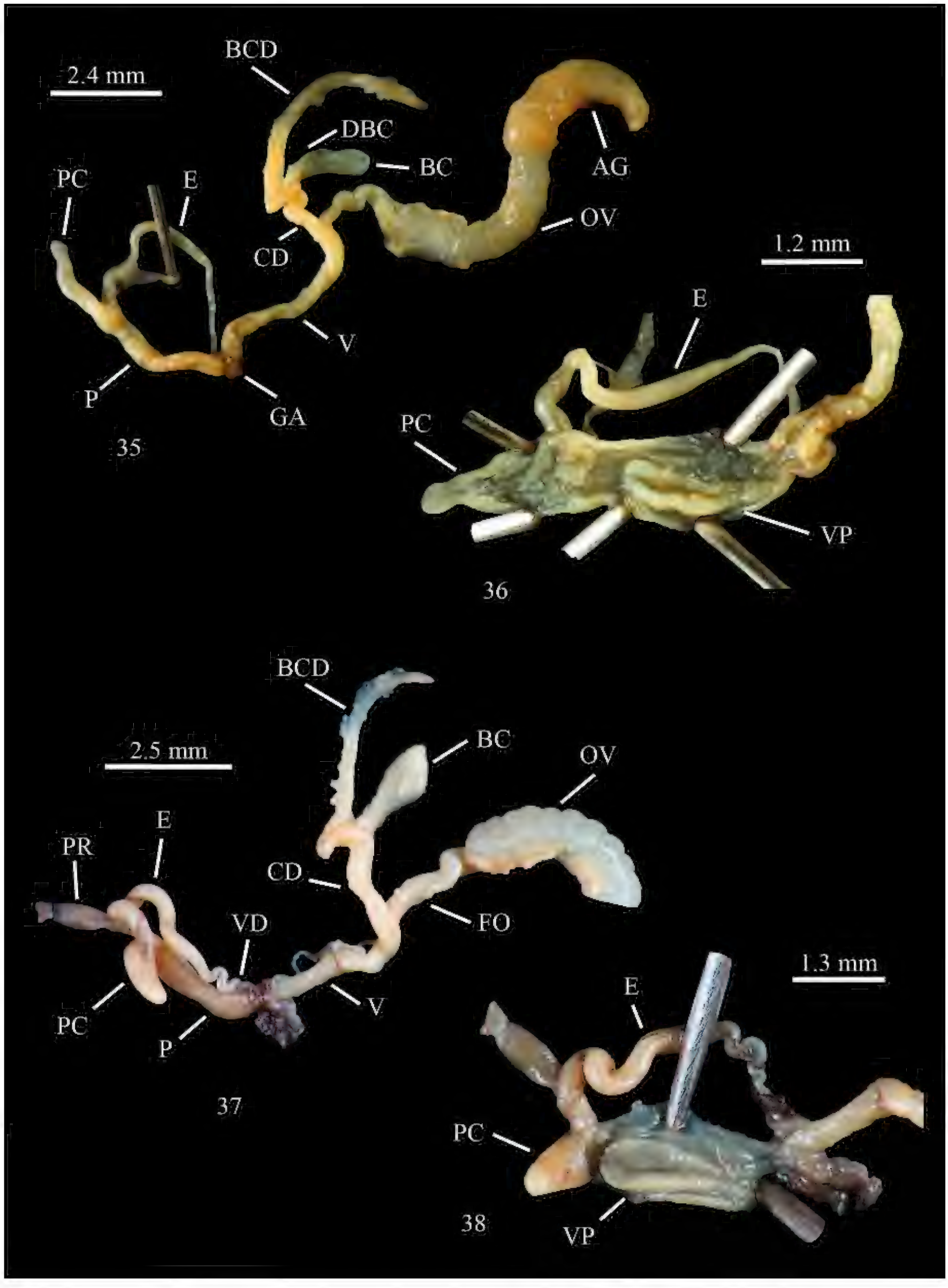


Figures 24–27. Genitalia of *Albinaria* (*Albinaria*) *brevicollis maltezana*, Konoupi Islet, islads group of Astypalea, Greece. Fig. 24. Genitalia. Fig. 25. Internal structure of penis, same specimen of figure 24. Fig. 26. Genitalia. Fig. 27. Internal structure of penis, same specimen of figure 26.

Fig. 28. Shell of *Albinaria* (*Albinaria*) *brevicollis* cf. *sica*, Dhragospilia, Astypalea Island, Greece, H: 15.2 mm, D: 3.2 mm. Fig. 29. idem, H: 16.45 mm, D: 3.15 mm.



Figures 30–34. Parietum, Palatum and Clausilium of *Albinaria* (*Albinaria*) *brevicollis* cf. *sica*, Dhragospilia, Astypalea Island, Greece. Fig. 30. Parietum. Fig. 31. Parietum. Fig. 32. Palatum. Fig. 33. Palatum. Fig. 34. Clausilium, same specimen of figure 32.



Figures 35–38. Genitalia of *Albinaria* (*Albinaria*) *brevicollis* cf. *sica*, Dhragospilia, Astypalea Island, Greece. Fig. 35. Genitalia. Fig. 36. Internal structure of penis, same specimen of figure 35. Fig. 37. Genitalia. Fig. 38. Internal structure of penis, same specimen of figure 37.

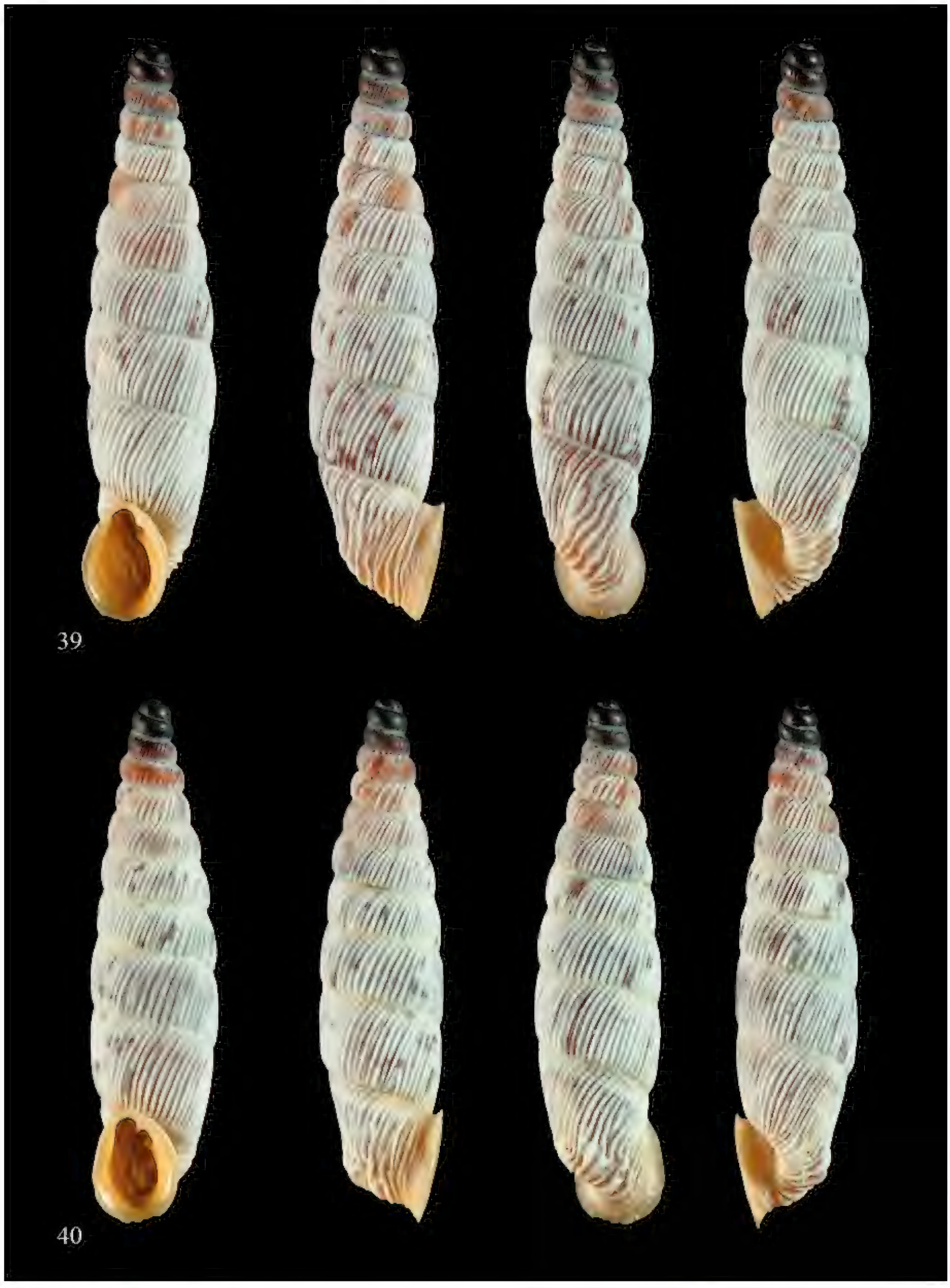
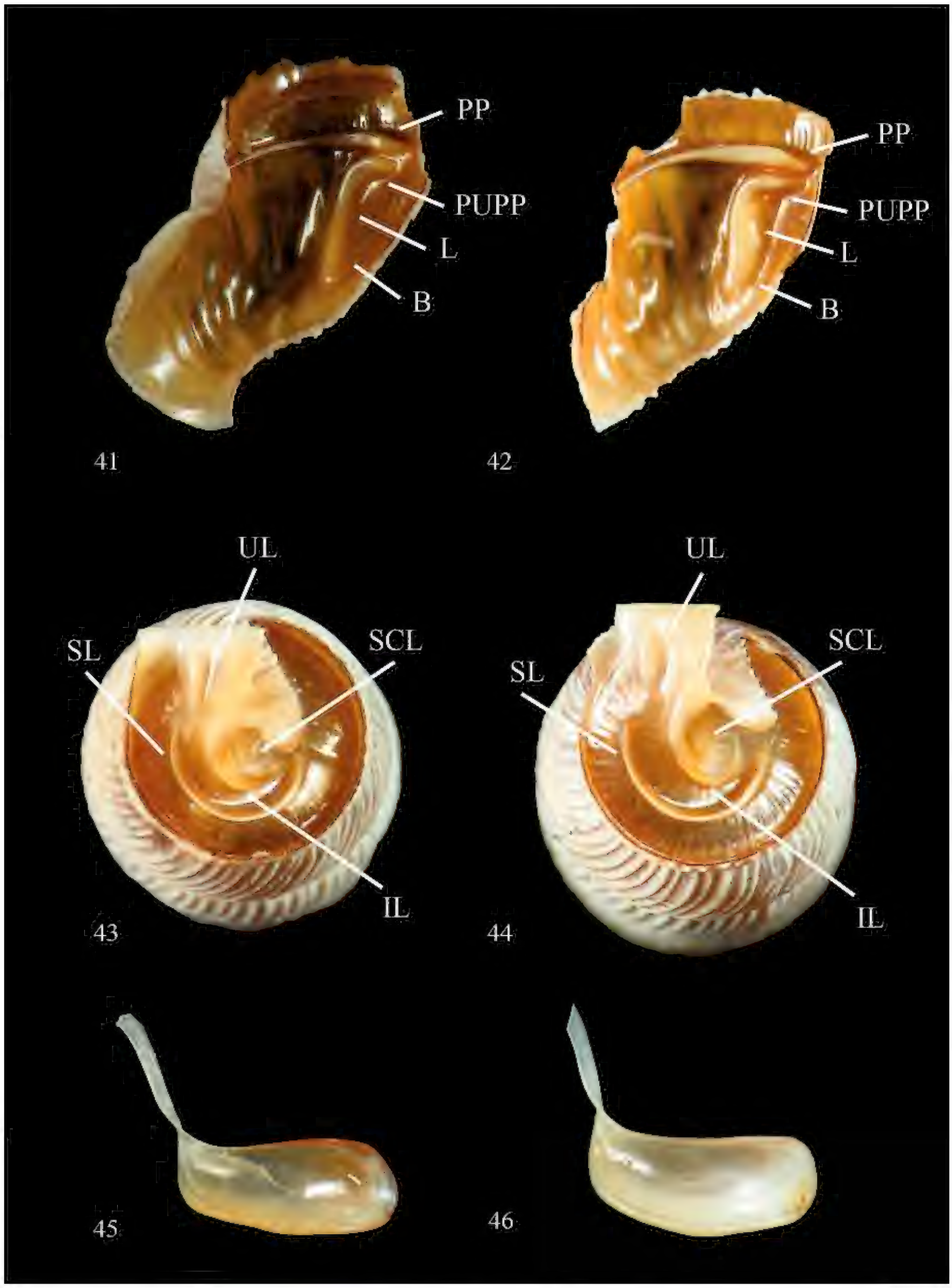
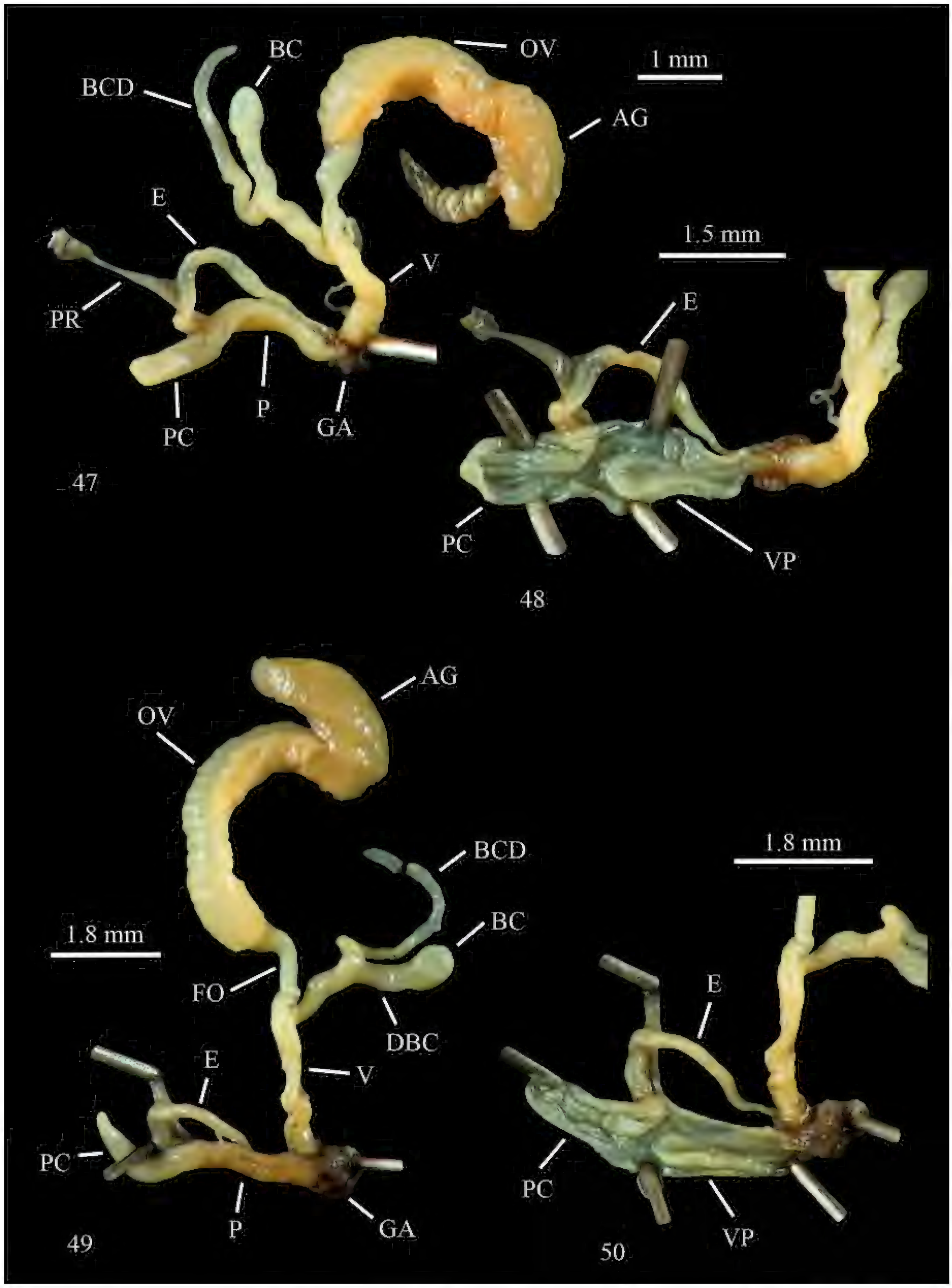


Fig. 39. Holotype of *Albinaria* (*Albinaria*) *brevicollis granoi* n. ssp., Pachia Ammos, Astypalea Island, Greece, H: 12.9 mm, D: 2.9 mm. Fig. 40. Paratype *A.* (*A.*) *brevicollis granoi* n. spp., idem, H: 12.7 mm, D: 3 mm.



Figures 41–46. Parietum, palatum and clausilium of *Albinaria* (*Albinaria*) *brevicollis granoi* n. ssp., Pachia Ammos, Astypalea Island, Greece. Fig. 41. Parietum. Fig. 42. Parietum. Fig. 43. Palatum. Fig. 44. Palatum, same specimen of figure 42. Fig. 45. Clausilium. Fig. 46. Clausilium, same specimen of figure 44.



Figures 47–50. Genitalia of *Albinaria* (*Albinaria*) *brevicollis granoi* n. ssp., Pachia Ammos, Astypalea Island, Greece. Fig. 47. Genitalia. Fig. 48. Internal structure of penis, same specimen of figure 47. Fig. 49. Genitalia. Fig. 50. Internal structure of penis, same specimen of figure 49.

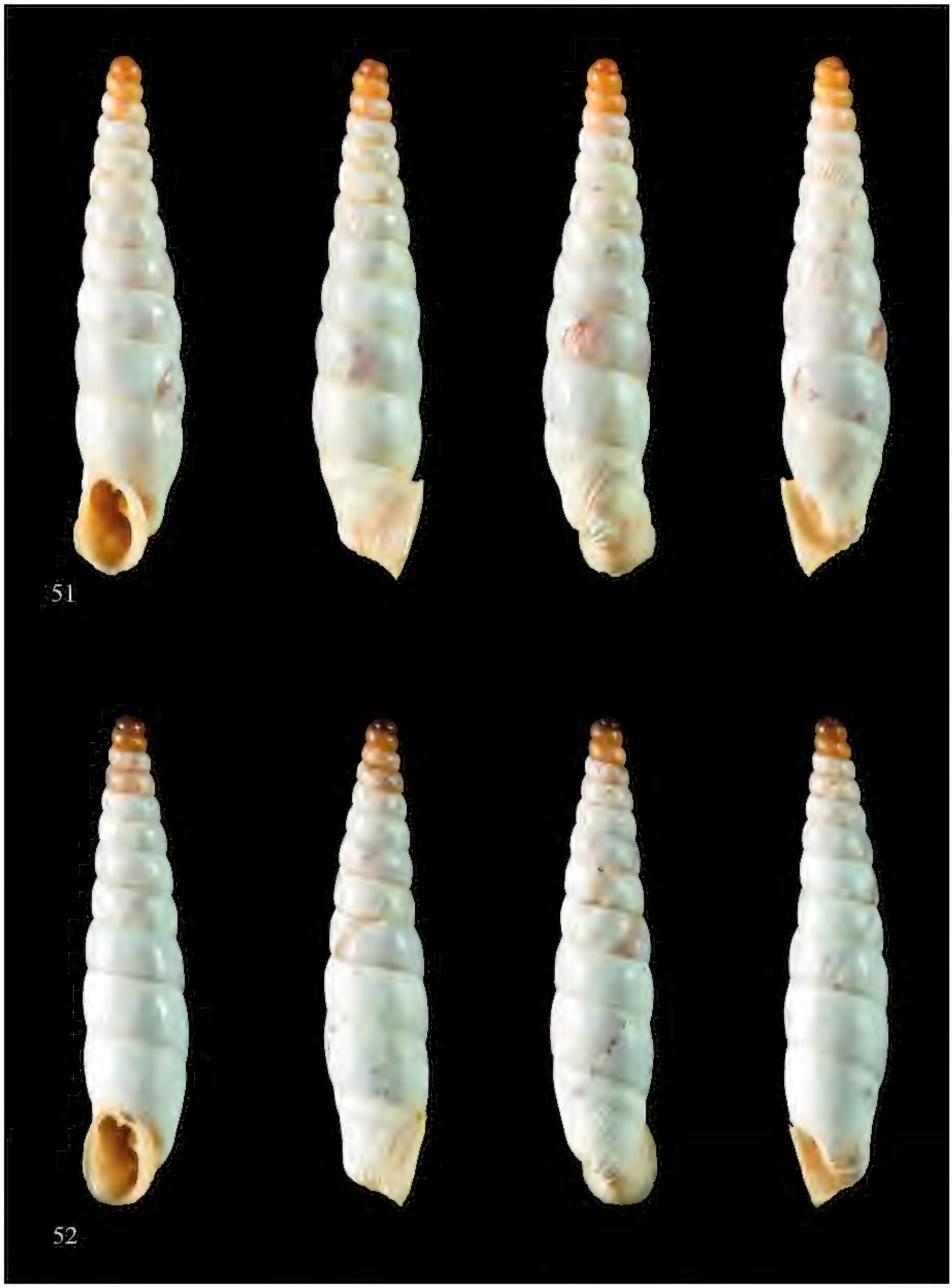


Figure 51. Holotype of *Albinaria* (*Albinaria*) *brevicollis cristinae* n. ssp., Ofidoussa Islet, islands group of Astypalea, Greece, H: 14.5 mm, D: 3.1 mm. Fig. 52. Paratype, *A.* (*A.*) *brevicollis cristinae* n. spp., idem, H: 14.7 mm, D: 3.1 mm.

VARIABILITY. Height: 12.7–15 mm (mean 13.9 mm), D: 2.8–3.1 mm (mean 2.9 mm), whorls: 10–11.75 (mean 10.5), R2 (n = 15): 9–17 (mean 14). Dorsal keel about as high as basal keel or slightly stronger; upper lamella reaches or does not reach the spiral lamella; lower part of lunella with a rudiment of basalis (Figs. 40–44).

Dimensions of the genitalia (Figs. 47–50): P: 1.75 mm, E: 2.6 mm, PC: 1.7 mm; vagina: 1.85 mm, CD 1.25 mm, DBC+BC 1.46 mm, BCD: 3.2 mm.

ETYMOLOGY. Named in honour of Mauro Grano (Rome, Italy), Italian herpetologist, who together with his colleague Cristina Cattaneo collected this new subspecies.

DISTRIBUTION. Mountain ridge North-West Astypalea Island.

REMARKS. The smaller dimensions and the rounded and slender apical and subapical whorls differentiate this subspecies from the other ribbed subspecies *A.* (*A.*) *brevicollis brevicollis* from the Rhodes Island, *A.* (*A.*) *brevicollis telensis* K.L. Pfeiffer, 1955 from the Tilos island, *A. brevicollis theodori* K.L. Pfeiffer, 1955 from the island of San Theodoros. Finally, *A. brevicollis granoi* n. ssp. differs from *A. brevicollis maltezana* for the smaller dimensions, lower number of whorls, greater number of ribs and less developed dorsal keel and upper lamella.

Albinaria (Albinaria) brevicollis cristinae n. ssp.

TYPE LOCALITY. Ofidoussa Islet, west of Astypalea Island, Dodecanese Archipelago, Greece.

TYPE MATERIAL. Holotype (Fig. 51): Ofidoussa Islet, 36°33'12.38''N 26°08'23.29''E, 82 m, legit M. Grano and C. Cattaneo, 18.VIII.2015, (MCZR-M-TYPE 00251/H). Paratypes: idem, 3 shs (CL 298–300; Fig. 52 shells CL 300).

DIAGNOSIS. *Albinaria brevicollis cristinae* n. ssp. is characterized by: slender, white shell; whorls convex and smooth; only on the last part of the last whorl there are thin and dense striae that reach the suture; dorsal keel prominent, upper lamella does not reach or reaches the spiral lamella.

DESCRIPTION OF THE HOLOTYPE (Fig. 51). Slender conical shell, only the last whorl tapering downwards; H: 14.5 mm, D: 3.1 mm, with 2.5 apical whorl light brown, the other 9 whorls white with few brown spots; whorls convex and smooth, only the first three subapical whorls have weak striae and the last part of the last whorl shows very thin and dense striae that reach the suture; sutural bulge marked; basal keel distinct, dorsal keel stronger and shorter, convergent with basal; peristome detached; oval mouth, inside yellowish white. The upper lamella reaches the spiral lamella, the inferior lamella low, subcolumellar lamella not visible in oblique vision; lunella dorsal-dorsolateral in position; principal plica and posterior upper palatal plica ending dorsolaterally.

VARIABILITY. Height: 14–16.4 mm (mean 14.9 mm), D: 2.75–3.1 mm (mean 3.0 mm), whorls: 11.25–11.5; subapical whorls without striae in the three paratypes. The upper lamella does not reach (2 shs) or reaches the spiral lamella (2 shs); inferior lamella low or moderately high (Fig. 52).

ETYMOLOGY. Named in honour of Cristina Cattaneo (Rome, Italy), Italian botanist and herpetologist, who together with her colleague Mauro Grano collected this new subspecies.

DISTRIBUTION. Known only from type locality: Ofidoussa Islet.

REMARKS. *Albinaria brevicollis cristinae* n. ssp. is somewhat similar to *A. brevicollis heracleensis* (O. Boettger, 1883) (Syn.: *A. brevicollis karavica* Fuchs & Käufel, 1936) from the Karavi Nisa Islet (64 km southeast of Ofidoussa).

Albinaria brevicollis cristinae n. ssp. is distinguished by *A. brevicollis heracleesnsis* for: the shorter and most prominent dorsal keel; the slightly more convex whorls, the thinner and more dense striae on the last part of the last whorl; more developed upper lamella (Boettger, 1883; Fuchs & Käufel, 1936; K.L. Pfeiffer, 1955; Nordsieck, 1999).

ACKNOWLEDGMENTS

We are grateful to Hardmut Nordsieck (Forschungsinstitut, Frankfurt am Main, Germany) for helpful comments on the manuscript.

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Biodiversity Journal, 2019, 10 (4): 569–576 https://doi.org/10.31396/Biodiv.Jour.2019.10.4.569.576

Characterization of gypsy moth *Lymantria dispar* (Linnaeus, 1758) (Lepidoptera Lymantriidae) eggs in Cork oak forests of the Kabylie region (Jijel-Algeria)

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ABSTRACT Algerian oak forests, extending over the entire northern littoral region, are attacked episodically by many defoliating Lepidoptera, of which *Lymantria dispar* (Linnaeus, 1758) (Lepidoptera Limantriidae) is the most widespread. In our study we aim to highlight the action of the changes of trophic factors on the dynamics of the population of *Lymantria dispar* through field inventory methods. Namely: the eggs counting by the line-transect method, picking of the eggs, control of eggs quality. Carried out on two stations in the Kabylie region (Jijel), in the forest of Béni Ider El M'sid (Taher) and that of the Ouled Djendjen Canton Boudouda (Texanna). The obtained results show that Texanna station is distinguished by a very high non-viable egg rate compared to Taher station, this is caused by the difference between the phenology of *Lymantria dispar* and the host tree which is affected in its foliage by various factors whose altitude is one of these factors.

KEY WORDS Algeria; defoliation; egg mass; Jijel; *Lymantria dispar*; Oak cork.

Received 20.06.2019; accepted 25.10.2019; published online 30.12.2019

INTRODUCTION

Forests are the main shelter for terrestrial biodiversity, as they represent an important link for the successful integration of biodiversity conservation and socio-economic development (Liang et al., 2016). The Mediterranean forests are considered as biodiversity hotspots (Myers et al., 2000; Quézel & Médail 2003; Mendoza-Fernández et al., 2010; Vessella et al., 2017; Tabet et al., 2018) , which is of major interest in the context of biodiversity conservation (Quézel et al., 1980). Forest dieback is becoming increasingly important due to long and intense periods of drought (Camarero et al., 2015; Lloret & Kitzberger, 2018). The cork oak is an endemic species which characterizes the western Mediterranean zone, from the economic point of view it is the most important forest essence in North Africa, and it has important interests for the ecological and socio-economic balance (Piazzetta, 2006).

Cork forests in Algeria suffered a severe degradation which was the cause of a loss of almost half of the original surface of 450,000 Ha (Chenel, 1951) and only 229,000 Ha remain that are considered productive (Aouadi et al., 2010). Consequently, the strong degradation of this forest species has

affected its productive capacity, which constitutes a significant economic potential, and which has greatly reduced in recent years (see Musset, 1954 and Roula & Chouial, 2005).

Among biotic Enemies, which may be a cause of degradation of Mediterranean forests, *Lymantria dispar* (Linnaeus, 1758) (Tae Hwa Kang et al., 2017) is a butterfly of the Lymantriidae family (Nierhaus-Wunderwald & Wermlinger, 2001). This gypsy moth is a pest of forest species (Gray, 2010), polyphagous and phyllophagous that lives on more than 300 species of trees and shrubs (Duan et al., 2011); prefers oak leaves (Nierhaus-Wunderwald & Wermlinger, 2001). The geographical distribution of *L. dispar* is remarkable: the Gypsy Moth, originates from Korea and Japan (Sparks et al., 2013; Contarini et al., 2016), has reached Scandinavia and the Mediterranean area passing through several countries including, among others, China, Afghanistan and Iran and Spain in Europe (Mecellem & Chakali, 2016). Fagaceae are widely threatened by this insect in Algeria, Morocco and Tunisia (Fraval, 1989; Villemant, 2006).

The very first observation of *L. dispar* infestations was recorded in the Edough Forest in the nineteenth century (Mecellem & Chakali, 2016). Since then, it has spread over all the oak forests of northern Algeria, particularly in the oak forests of Babors, in the forest of Blida and the forests of Kabylie. It is also present in the majority of West-northern oak forests (Laaribya et al., 2010; Mecellem & Chakali, 2016).

This insect is the origin of the disturbances of the pollination of the oak species, which endangered its production of the glands and the regeneration (Zeraia, 1988). The immediate nuisance of *Lymantria dispar* is the weakening of the tree which becomes vulnerable to the attack of xylophagous insects and lignivorous fungi (Fraval, 1989).

The forestry services pointed out that in 1977, *Lymantria dispar* invaded a very large forest area affecting the whole of Jijel wilaya, between 1984 and 1989 a second major infestation was recorded in the region, during which cork oak suffered significant defoliation (Villemant, 2006).

The present study is based on the effect and action of the trophic factor on the population dynamics of *Lymantria dispar*. The interest of the current research aims at the enumeration and characterization of the insect's eggs in its natural environment, whose main objective is to try to deduce the infested zones.

This study constitutes a support for the decision for a more rational management of infested species in the Jijel region and to know the priority areas for interventions.

MATERIAL AND METHODS

Study area

In the sub-humid bioclimatic domain of the Jijel region, two stations were chosen, the first being the Ouleds Djendjen forest, Boudouda (Texanna), a *Quercus suber* forest on a siliceous substratum, and at an altitude of 850 m south of the wilaya of Jijel and the second is the forest of Beni Ider, El M'sid (Taher), a high forest *Quercus suber* anchored in a shallow schistose substrate at an altitude of 570 m, south-west of Jijel.

The methodology followed is classic and is divided into three main phases which are detailed below, after choosing 15 trees as samples for each of the stations.

Phase of egg masses counting

The line-transect method has been adopted in this work, it consists of randomly establishing at the center of the sampling plot a series of fixed routes, the latter is located in the middle of the plot along these routes by moving at a regular interval to count all egg masses. The entire tree is taken as a sampling unit, the count of the egg masses is performed on 15 selected trees on a systemic linear altitudinal transect, the objective is to avoid edge effects (Chakali & Ghelem, 2008). Once spotted, the clutches are dated and marked with numbered tags.

Practically, we proceed to the counting of the egg masses visually, from the ground. For each sample tree, we do counting on the first meter of the trunk, , and one meter around the tree. After exploring the trunk and its crevices, the observer looks through the scaffold branches and shoots, choosing systematically the branch whose diameter is the smallest at each junction (Fraval, 1981; Zeraia, 1988; Laaribya et al., 2010).

For each tree two counts are made by two people in order to better quantify and confirm the number of lepidopteran egg masses. The majority of females lay their eggs on the lower parts of the branches to better protect themselves against natural enemies, including birds.

Egg masses collection

We measure the diameters of the clutches masses using a vernier caliper. These egg masses are collected and put into perforated bags to ensure aeration of the eggs. In the laboratory, with a binocular loupe, the eggs counting is done for each mass of eggs. The technique used in the counting of nonviable eggs (unhatched, flattened and parasitized) as well as the removal of eggs from their fluff is that proposed by Fraval (1989).

Control of Lymantria dispar *eggs*

After a first check (removal of fluff, counting of unhatched eggs, hatched, parasitized, dry, and broken eggs), we were able to determine the different states of lepidopteran eggs at the two study stations.

RESULT AND DISCUSSION

The analysis results of the collected data are organized in four steps, namely:

1) comparison of the number of eggs and the diameter of the egg masses between the two stations,

2) relationship between the number of eggs and the diameter of the egg masses of the two sites,

3) the results relating to the quality of the eggs of *Lymantria dispar*,

4) place of preference for laying eggs.

Number of eggs and diameter of egg masses

Statistical analysis showed no significant difference in the number of eggs for the two stations studied ($P > 0.05$, $F3.92 = 2.367$). On the other hand, a significant difference is recorded for the diameter of the clutches ($P = 0.039$, $F3.92 = 4.363$). The average diameter of lepidopteran clutches at the Taher station is larger than that of the Texanna station (Table 1).

Relationship between the number of eggs and the diameter of the egg masses

A partial correlation model was adopted to highlight the nature of the link between the two parameters number of eggs and the egg masses diameter for each station. These are positive and significant correlations but are considered average (Table 2).

Quality of the Lymantria dispar's eggs

At the two stations selected, the percentage of different categories of nonviable eggs (flattened, dry and parasitized) compared to viable categories is very important (Fig. 1).

Clutches are more exposed to the oophagous parasite, *Ooencyrtus kuvanae* (Howard, 1910) (Hymenoptera Encyrtidae) and predator-dismantlers such as *Dermestes lardarius* Linnaeus, 1758 (Coleoptera Dermestidae). The action of this predator is limited to 2 to 3 weeks after the laying period of *Lymantria dispar* (Luciano & Prota, 1984).

The results revealed a significant difference ($P < 0.001$, $\chi 216.27 = 53.589$) between different types of eggs (Table 3). The rate of viable eggs is higher (73.23%) at the Texanna station compared to Taher station (57.95%). The rate of parasitized eggs, unlike viable eggs, is higher in the Taher station (27.64%) than in the Texanna station (18.53%). The remaining eggs (unhatched and flattened) accounted for only 8.24% and 14.4% at both Texanna and Taher stations, respectively (Fig. 2).

Place of preference for laying eggs

Statistical analysis showed no significant difference in the preference for laying eggs ($P > 0.05$, $\chi 23.84 = 0.633$) (Table 4). The female has no preference with regard to the place of deposit of her eggs on the tree.

The same observations can be noted for the two Taher and Texanna study areas, and despite the difference in certain factors, in particular altitude, the variation in environmental conditions is little.

On the trees, the majority of the clutches are deposited on the trunks where population density of the insect is low. However, during outbreaks, the number of laying increases, and the insect lays and places its eggs all over the tree.

Parameters / Station	Number of eggs	diameter of laying-eggs (mm)
Taher	302.72 ± 24.84a	16.16 ± 041a
Texanna	362.03 ± 29.49a	14.43 ± 0.72b
$F_{3.92}$	2.367	4.363
P	0.127	0.039*
d. d. l.	1 / 118	1 / 118
n	60	60

Table 1: Number and diameter of laying-eggs in both Taher and Texanna stations (Mean ± ES). The averages followed by different letters in the columns are significantly different (ANOVA; * P <0.05). F: calculated factor. P: probability, d.d.l .: degrees of freedom. v1 = 1, v2 = 118. n: number of repetitions.

Parameters		**Number of eggs**	
	Stations	***Taher***	***Texanna***
Diameter of egg masses	***Taher***	r = 0.32 (P = 0.013)*	
	Texanna		r = 0.335 (P = 0.009)**

Table 2. Correlation coefficients (r) between the number of eggs and the diameter of the egg. * P < 0.05. ** P < 0.01.

Parameters / Stations	Viable eggs	Unhatched eggs	Flattened eggs	Parasited eggs	**Total number of eggs**	Chi-square test ($\chi 2$)
Taher	499	70	54	238	861	$\chi^2 = 53.589$ $v = 3$ P = 0.000*
Texanna	818	60	32	207	1117	
Total number of eggs	1317	130	86	445	1978	

Table 3. Statistical analysis of egg quality. (v: degrees of freedom, *P < 0.001).

Parameters / Stations	Number of egg masses on trunk	Number of egg masses on main branches	Total	Chi-square test ($\chi 2$)
Taher	37	59	96	$\chi^2 = 0.633$ $v = 1$ P = 0.426
Texanna	42	53	95	
Total	79	112	191	

Table 4. Place of preference for depositing the egg masses. (v: degrees of freedom).

DISCUSSION

The issues addressed in this study provide additional information on the behavior of *L. dispar* in its natural environment.

The abundance of the clutches observed during our census shows the females' high reproductive success of *L. dispar*. The presence of unfertilized eggs in large numbers in the egg-laying and eggs undergoing high mortality is probably due to poor trophic conditions (Fraval, 1989), the environmental conditions affect the periods and durations of *L. dispar* development phases in its natural environment (Hlasny et al., 2015). This desynchronism between the phenology of the *L. dispar* and the host tree is undoubtedly the determining factor of the presence of unfertilized eggs, since the larval development phase is more sensitive to sudden environmental changes, which may have a spreading of the infestation period of 3 to 4 years in the case of coastal werate trees (Fraval, 1989; Khous & Demolin, 1997; Mecellem & Chakali, 2016).

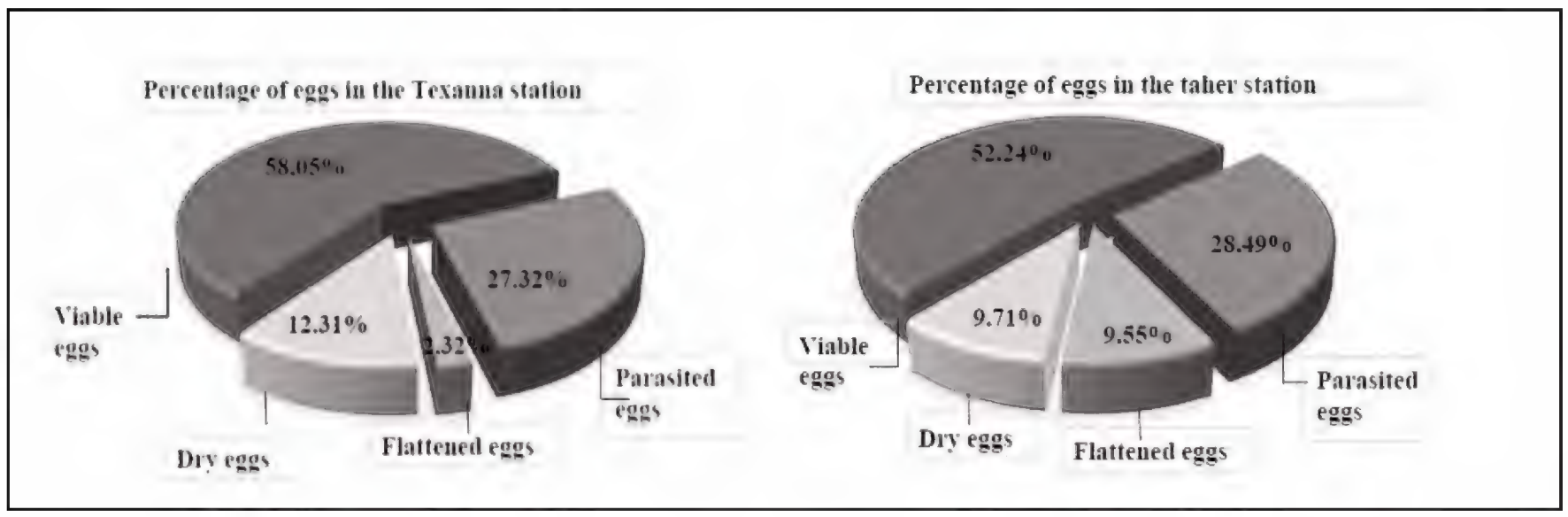

Figure 1. Percentage of different eggs categories at both stations (Taher and Texanna).

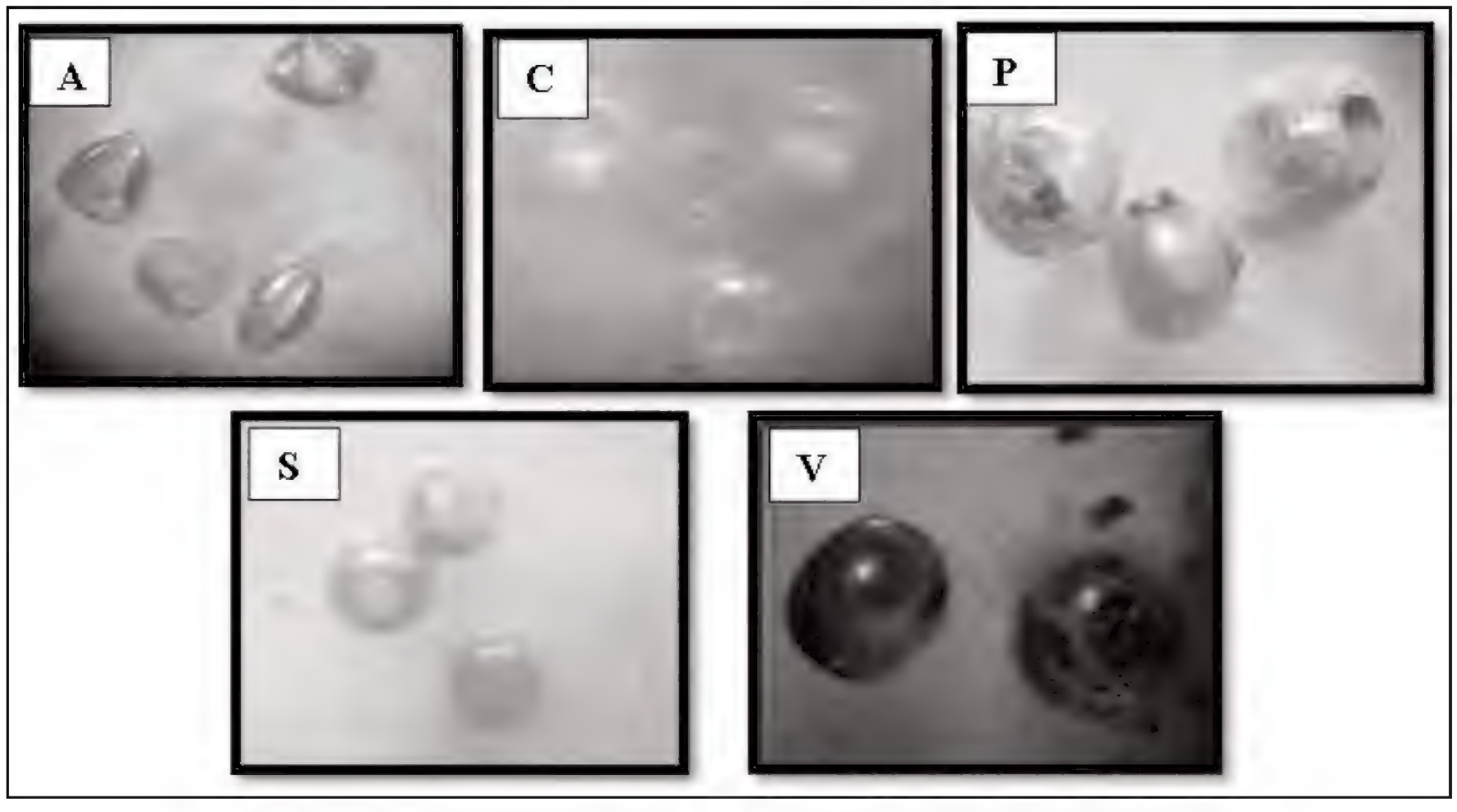

Figure 2. Categories of *Lymantria dispar* eggs providing information on the causes of death. (V: viable, P: parasited, C: broken, A: flattened (not fertilized), S: dry (dead embryo).

In most cases, it should be assumed that natural enemies are sufficient to reduce defoliator populations. *Ooencyrtus kuvanae* is the only oophagous parasite of *Lymantria dispar* recorded at the level of the two stations but also the predators-deniers such as *Dermestes lardarius*, among others, were present and did important damage to the bombyx's eggs. This result is confirmed by the work of Morsli (2008) and also by those of Basir et al. (2005).

Large foci have been localized at medium altitude (570 m) at the level of El M'Sid (Taher) cork oak. Defoliation at the level of the plains is characterized by their extension on large surfaces contrary to high altitudes as in the case Boudouda (Texanna 850 m), at the level of high altitudes the defoliation affects the forest massifs successively during considerable periods of time (Zamoum et al., 2014).

Lymantria dispar is a very dynamic species, able to live at the expense of forests and very varied species (Duan et al., 2011) under very different climatic conditions (Mecellem & Chakali, 2016). In eastern Algeria, in the case of delayed budding of the cork oak or when the clutches are distant from the host plant, the larvae can continue a part of their development on some tree species (Stoyenoff et al., 1994; Ouakid et al., 2001; Morsli, 2008). The other cause that facilitates the frequent attack and the installation of this pest on the forests of the study area is their weakening by the human intervention and consequently the frequent fires (Zamoum et al., 2014).

The vast operations of struggle put into action today do not prevent the increase of the areas subjected to the defoliation of this lepidoptere. The action of this gypsy moth can result in the weakening of the attacked tree subjects, the latter being subjected to a strong attack of xylophagous and lignivorous insects and parasites which can in their turn weaken the affected stands.

Lymantria dispar is the best-known defoliator and the most widespread defoliator in cork oak forests, causing spectacular defoliation periodically, its attack is made according to an altitudinal gradient where the infested species are more and more green with late growth, suitable for the completion of its development cycle and to ensure its sustainability.

Apart from any parasitic causes of this defoliator, the latter showed a good dynamism which is proved by the good progress of the laying on the majority of the compartments of the tree. This translates the preference of the gypsy moth of this species cork oak which offered him optimal development.

Actions to control this type of insect pest have priority and urgency for affected stands, especially forests with high susceptibility to caterpillar attack.

ACKNOWLEDGEMENTS

My particular thanks go to Mr Kerris Tayeb for helping us to obtain a permit to work in the entomology laboratory of the regional forest research station of Jijel. I also thank very much Mr Abdelkrim Arar of the university of M'sila in Algeria and Slimane Tabet of the university of Mila in Algeria for their precious help in carrying out this work.

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Biodiversity Journal, 2019, 10 (4): 577–588 https://doi.org/10.31396/Biodiv.Jour.2019.10.4.577.588

Bioecological study of parasitic complexes of aphids in North-West Algeria

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ABSTRACT

Myzus persicae (Sulzer, 1776) (Hemiptera Aphididae) is the most significant aphid pest of peach trees. Chemical control of this species is a quick and simple method to prevent the development of this pest, however, the massive use of these chemicals poses potential health and environmental risks. This study proposes an alternative biological control approach based on the use of parasitoids to reduce aphidian populations. The study, which we undertook over the course of three years, allowed us to observe almost the same species of parasitoid (Hymenoptera Braconidae) on the vegetable crops taken into exam in the study. However, some species were considered to be absent in the region. Others appeared only during the second and last year of study as *Aphidius funebris* Mackauer, 1961, *Trioxys angelicae* Haliday, 1833 and *Praon exsoletum* Nees, 1811. This study showed total dominance of *A. matricarae* Haliday, 1834 with very high parasitism (values of 61%, 54% and 78% during 2012, 2013 and 2014, respectively) followed by *Lysephlebus testaceipes* Cresson, 1880.

KEY WORDS *Myzus persicae*; biological control; dominance; parasitoids; aphidian populations.

Received 26.08.2019; accepted 25.10.2019; published online 30.12.2019

INTRODUCTION

In Algeria, market gardening is the second crop after that of cereals. It occupies an area of more than 330,000 Ha with an estimated production of 8.5 million tons in 2013 (F.A.O, 2013). As with most crops, vegetables are confronted with various phytosanitary problems, leading to economic losses of up to 100%.

Nowadays, in addition to the phytophagous insects known for the importance of their damage to vegetable crops, especially under greenhouse shelters such as whiteflies, moths and thrips, we find other pests more dreadful on these crops and espe-

cially on the greenhouse pepper, in this case, the aphids (Blancard, 1988; Gillespie et al., 2002).

Aphids are more than ever a concerning pest for many crops. They affect vegetable crops as well as field crops, orchards or floral crops (Norozou, 2013). These aphids, which settle early on crops, have an exceptional multiplication rate. Their biological characteristics make them formidable pests and they are the cause of numerous damage, at all stages of the cultures (Bouhroua, 1987).

Particularly, *Myzus persicae* (Sulzer, 1776) (Hemiptera Aphididae) is the most significant aphid pest of peach trees and it is also a vector for the transport of plant viruses.

Control of these aphids is more feasible through the application of synthetic insecticides that can limit their populations to a tolerable threshold (Lopez et al., 2012). This means of control can lead to several harmful effects such as the reduction of natural enemies, the appearance of resistant strains in pests, etc. Aphids have developed resistance to chemical pesticides (Riba & Silvy 1989; Foster et al., 2003; Wang et al., 2007). However, many studies aimed at biological control aim at exploiting and valuing the action of many natural enemies. This method implies a perfect knowledge of the biology of the pest in question and that of its natural enemies (Estevez et al., 2000).

Thus, the agricultural world has been pushed to adapt chemical control to minimize the number of chemical treatments. Significant development of biological control is currently the most advocated. This method of control aims at the effective use of the potentialities of auxiliary fauna, whether they are predators or parasitoids against aphids.

The growing interest in conservation biological control measures underscores the need to study the diversity and phenology of populations of aphidiphagous helper insects (Bouhroua, 1991).

Biological control in greenhouses is rapidly growing worldwide because of the advantage that a closed environment has for applying biocontrol agents (Boissard et al., 2008; Driesche et al., 2008; Lopes et al., 2009; van Lenteren, 2000a cited by Norouzi, 2011). In some systems, biological control has good potential for replacing chemical methods of arthropod pest control (Gillespie et al., 2002; van Lenteren, 2000b cited by Norouzi, 2011).

Several families of insect predators and parasitoids can control aphid populations, mainly ladybugs (Coleoptera Coccinellidae), syrphids (Diptera Syrphidae), chrysopes (Neuroptera Chrysopidae) and micro-Hymenoptera belonging to the family Braconidae and Aphelinidae (Lyon, 1983; Boivin, 2012; Lopes et al., 2012). The latter restrict aphid populations (Laamari et al., 2011). Parasitoids include the Hymenoptera of the family Braconidae and the subfamily Aphidinae. It encompasses about 400 species worldwide (Laamari et al., 2011). Some of these species are solitary and aphid-specific parasitoids (Kavallieratos et al., 2001; Aslan et al., 2004).

The Aphidiidae family is the most represented among the parasitic activity of aphid parasitoid species (Darsouei et al., 2011; Hemidi et al., 2013). Most of these species belonging to this family are koinobiont endoparasitoids of aphids (Kavallieratos et al., 2001; Aslan et al., 2004; Boivin et al., 2012). According to Akhtar et al. (2011), these Aphidiidae are known from all major habitats in the world, especially in the temperate and subtropical zones of the northern hemisphere.

According to Bouhraoua (1991), numerous authors throughout the world confirm that aphids are attacked in the field and in the greenhouse by a very large number of entomophagous species. They often succeed in completely eliminating the colonies of these aphids on cultivated plants. At present, in our country, the list of Aphidinae Hymenoptera has reached 32 species (Ghelamallah, 2016, 2018).

Indeed, it is very important to apply integrated pest management strategies in our pest management strategies that promote the exploitation of the action of many natural enemies and the use of selective chemicals, without eliminating the action of the auxiliary fauna. This approach should be based on a thorough knowledge of the population dynamics of the pest in question and of its parasitoid fauna (Ghelamallah, 2016).

It is with this vision that our research work focuses on the knowledge of the bioecological parameters intervening in the regulation of the aphidian populations by using auxiliary fauna in order to preserve the balance of the agro-systems, thereby minimizing the use of insecticides.

Research carried out in the Mostaganem region deals with the monitoring of the population dynamics of *Myzus persicae* over a period of three years (2012–2014). They also make it possible to develop an exhaustive inventory of their natural enemies

with an assessment of the impact of different abiotic factors (temperature in this case) on the biotic regulation of aphid populations (parasitoids and predators). This work also made it possible to study the dynamics of the populations of this auxiliary fauna over the same period of 3 years (2012–2014).

The main objective is to collect the information needed to develop biological control techniques, based in particular on parasitoid fauna. As a result, several parameters of the biology of the aphids, especially of *M. persicae* and its antagonists, have been studied.

MATERIAL AND METHODS

In this context, the auxiliary fauna of greenflies, an inventory of natural enemies of aphides was pursued, from 2011 to 2014, fot the experimental studies of the Department of Agricultural Sciences at the Mostaganem University, Algeria. As far as our study was enlarged to different farming sites in many localities in the province of Mostaganem in the north-western part of Mostaganem. All material has been collected by the first author.

For four consecutive years, from early January to early July, 300 leaves contain larvae of devastators that have been collected each weekto make an inventory of hymenopterous parasitoid species. To each sample, all the mommies found within the colonies of the studied green flies are collected and driven to the laboratory, then are separated and placed in labelled tubes and followed until the emergence of adult parasitoids. Once the emergence is obtained, these adults are conserved individually in micro-tubes containing a 90% of ethanol for a further identification.

The species mentioned in this study (Hymenoptara Braconidae) are: *Aphidius colemani* Viereck, 1912, *A. ervi* Haliday, 1834, *A. funebris* Mackauer, 1961, *A. matricariae* Haliday, 1834, *A. platensis* Brèthes, 1913, *A. transcaspicus* Telenga, 1958, *Binodoxysc angelicae* Haliday, 1833, *Diartiella rapae* M'Intosh, 1855, *Lysephlebus fabarum* Marshall, 1836, *L. testaceipes* Cresson, 1880, *Praon volucre* Haliday, 1833, *P. exsoletum* Nees, 1811, *Trioxys angelicae* Haliday, 1833.

Calculation formulas used rate of parasitism:

Tp = (Number of parasitized individuals / Σ of enumerated individuals) × 100.

RESULTS

The results obtained are statistically treated by the STATBOX PRO software and a comparison of the averages is performed on the Newman and Keuls test at 5%.

A factorial correspondence analysis (CFA) is performed using the Minitab 14 software. It is used to identify the effects of different months and years on relative abundances, parasitism and insect distribution in the foliar stage. Diagrams were also constructed to assess insect-specific abundance and years and months.

Relative abundance of inventoried parasitoids

In 2012, 8 species of Hymenoptera including seven parasitoids and one hyperparasitoids have been found. In contrast to the first year, we recorded new species during this period. These species are: *A. platensis*, *L. testaceipes*, *D. rapae*, and *L. fabarum*. On the other hand, we noticed the absence of two species *A. colemani*, *A. transcaspicus* during all the years of study. This absence can be explained by competition between species.

The differences revealed the presence of a newly established species in the study area. This is *A. platensis* with a relative abundance of 2% (Fig. 1).

In the first samplings, coinciding with February, the abundance of parasitoids recorded was very low except for the two species *A. matricariae* and *L. fabarum*. These two parasitoids are the most dominant with a rate of 61% for *A. matricariae* followed by *L. fabarum* with a relative abundance of 8%.

Comparatively, the other species identified, such as *A. platensis*, *A. ervi*, *L. testaceipes* and *D. rapae*, have values oscillating between 2% and 4%.

On the other hand, hyperparasitoids showed a presence at the end of the sampling period in May and early June with a rate of around 15%.

According to the factorial correspondence analysis, *A. matricariae* is very present during the three months of 2012 (Fig. 2) followed by *L. fabarum*, *D. rapae* and finally *L. testaceipes*.

The analysis reveals that the number of insects is very high in April compared to March and May respectively (Fig. 2).

During 2013, compared to 2012, we have identified two new species that appeared for the first

time in our experimental site. These are *P. volucre* and *B. angelica*. Thus, we observed the disappearance of the species *A. platensis*, and this is due to the predominance of certain species that settled in the site of the study (Fig. 3).

Relative abundances in 2013 were highly variable across species. The highest value was recorded in *A. matricariae* (54%) followed by *L. testaceipes* with 22%. The other parasitoids participate with relatively low abundances ranging from 5% in *L. fabarum* and *A. ervi* to 2% in *P. volucre* to 1% for *B. angelicae*. These values are relatively similar to those observed in the previous year, except for *B. angelicae* and *P. volucre*, where abundance appears to be lower (Fig. 4). For hyperparasitoids, we noticed a decrease of 8%.

From our results, we note that the number of *A. matricariae* is considerably higher than the other species (about 51% of the total).

Our observations show that the species *L. testaceipes* is present at 22% in relation to all the insects.

Factor analysis shows that the number of species recorded during the month of May is significantly higher than in April and June (Fig. 4).

In the fourth year of inventory (2014), only 9 species were observed, of which three species are listed for the first time: *T. angelicae*, *P. exsoletum* and *A. funebris*. The latter has marked its presence with a fairly appreciable rate of around 5% and will perhaps be the most dominant over time.

For relative abundance, we noticed a rather impressive increase in *A. matricariae* with a dominance which reached a maximum threshold of 78% (Fig. 5). For the remainder of the inventoried species, namely *A. funebris*, *L. testaceipes*, *D. rapae*, *A. ervi*, *P. volucre*, *T. angelicae* and *P. exsoletum*, their abundance is very low, ranging from 1 to 5% only.

Concerning hyperparasitoid, we observed that the rate remains almost identical to that of the previous year (10%).

During the year 2014, the number of *A. matricariae* is significantly higher than the other species with an estimated 78%.

The factor analysis of the correspondences shows that the month of April records a large number of individuals compared to the other months of the same year. This same analysis reveals that there is a close relationship between the number of *A. matricariae* and the month of April (Fig. 6).

It should be noted that the number of hyperparasitoid in 2014 is estimated to be 10% of the total number of insects surveyed.

Monthly relative abundance of different inventoried parasitoid species.

In 2012, according to figure 10, we note that April is the most favorable month for the development of parasitoids. This could be explained by the climatic conditions favorable to their development. We recorded an average temperature of 22 °C, which increases gradually to an average between 27 and 30 °C during the months of May and June. The density of aphid populations was high during this period.

The monthly proportions of each species showed a regular presence of *A. matricariae* during the various months of study and reached the maximum threshold during the month of April when the temperature inside the greenhouse is adequate to the reproduction of this species.

Aphidius ervi, *D. rapae* and *L. testaceipes* were observed only once during the study period. Thus, we observed that *A. platensis* appeared only during the month of May (Fig. 7), whereas certain species appeared only during the month of March, such as *L. testaceipes*.

Subsequently, a decrease in the level of parasitoid hymenoptera was recorded towards the end of April (Fig. 8).

The factorial analysis of the correspondences shows a positive correlation between the development of *A. matricariae* and the month of April (Fig. 8). However, the analysis also reveals a positive relationship between March and the same species. Our results also show that April is the right month for the development of all forms of insects. The number of insects in the month of May is smaller than in April when, apart from the *A. matricariae*, virtually no insects have been recorded.

In 2013, the monthly presence of the parasitoid species inventoried allowed to highlight their regular activity during the different months. We note in particular the predominance of *A. matricariae* in April and May, with the exception of the previous year where we noticed its presence during the month of June.

The monthly proportions from March to July of each species showed a regular presence of five

species, *L. testaceipes*, *L. fabarum*, *A. ervi*, *P. volucre* and *B. angelicae*, with a clear predominance of *L. testaceipes* from April to June (Fig. 9). Some species continue to appear until July. This presence during the summer can be explained by the temperatures favorable to the development of these parasitoid species.

Regarding the monthly relative abundance of hyperparasitoids, we recorded a significant presence of some species during May and June.

During 2013, the abundance of hymenoptera species is relative to the different months. Their factor analysis reveals that April is the ideal month for the development of *A. matricariae* and *L. fabarum* while the climatic conditions of June contribute to the growth of the number of individuals of *L. testaceipes* and *A. ervi* (Fig. 10).

During 2014, the monthly presence of the listed species revealed a constant activity of *T. angelica*, *A. funebris*, *L. testaceipes*, *P. exsoletum*, *D. rapae*, *A. ervi* and *P. volucre* during April. However, between February and June, we record the dominance of *A. matricariae* (Fig. 11). This high abundance of this parasitoid during the sampling period may be due to the favorable temperature, from 20 to 28 °C. It was in these thermal conditions that we recorded a relationship between the highest abundance of the insect during April and the average temperature of 24 °C.

The monthly proportions of hyperparasitoids showed a regular presence but earlier than those of last year. During this year, the appearance is more contrasted during April and May (Fig. 11).

During 2014, there was a significant decrease in the number of individuals of all species of hymenopterans recorded mainly during May and June. However, it is important to note that relative abundance is very high during April especially for *A. matricariae* (Fig. 12).

DISCUSSION

The relative abundance of aphidian parasitoids would differ from one year to the next (Kavallieratos et al., 2005). According to Andrade (2013), the different species of parasitoids can be influenced unequally by climatic variables. Depending on the year, the community may be dominated by one species or another. These very large fluctuations in abundance indicate the existence of an annual factor structuring these communities, possibly associated with climate variations and the host resource.

These biotic and abiotic variations have favored the appearance of certain species during each year at different rates of abundance: for example, the species *L. testaceipes* and *A. ervi*, are in total dominance of *A. matricariae*.

These results observed on the expansion and increasing predominance of this parasitoid are similar to those already observed by other authors, in particular Laamari et al. (2011) and Acheampong et al. (2012). Currently, we can consider that this species (*A. matricariae*) is one of the most efficient auxiliaries against the aphids in Algeria.

Aphidius matricariae is an important parasite of the green peach aphid, *Myzus persicae*, and 40 species of aphids belonging to 20 genera have been recognized as hosts (Rashki et al., 2009).

Throughout Algeria, Laamari & Stary (2013) mentioned that the parasitoid *L. testaceipes* occupies the second position after *A. matricariae*. It was able to develop 74 tritrophic associations. After being introduced to the South of France in 1973-1974 (Starý et al., 1988), it was introduced also in Spain (Baixeras & Michelena, 1983), Portugal (Cecilio, 1994) and, finally, north Africa, probably through the Straits of Gibraltar.

This species has been introduced into biological control against various aphid species in many parts of the world such as Australia (Carver, 1984) or the Mediterranean basin (Lopez, 2007). As well as *L. testaceipes*, which has parasitized 20 species of aphids predominantly harmful to crops, it can be used in biological control programs against these phytophagous plants (Laamari et al., 2011). According to Ouadah (2009), among the natural enemies of *Aphis gossypii* Glover 1877, the parasitoid *L. fabarum* plays an important role in limiting populations of this aphid on the cultivation of greenhouse bell peppers.

However, *A. matricariae* was the most dominant species, having already formed 57 tritrophic associations with 23 species of aphids found on 38 plant species (Laamari et al., 2011; Laamari & Stary, 2013).

The numerical importance of *Aphidius* and *Lysiphlebus* species can be attributed to their ability to adapt to different climatic conditions. According to Stary et al. (1975), species belonging to these genera are not very demanding from a climatic point of view. This is certainly what explains their

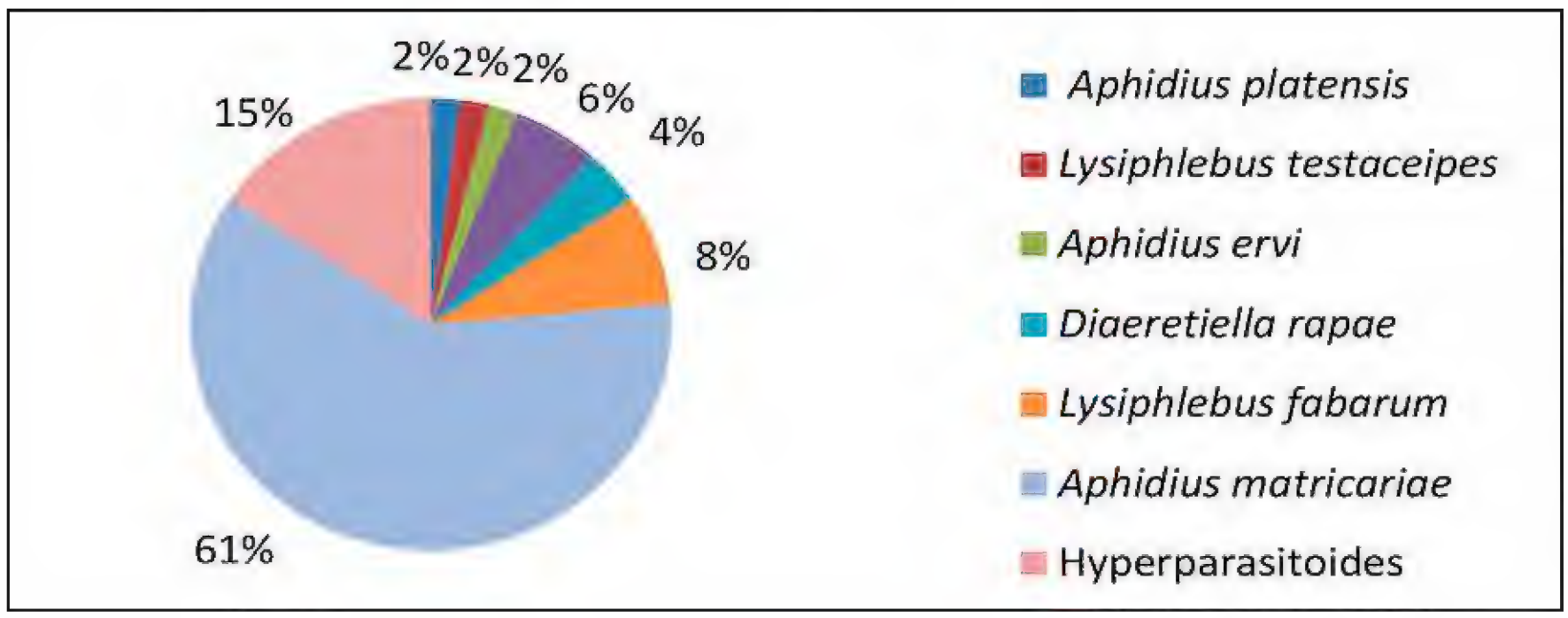

Figure 1. Relative abundance (%) of parasitoids taken during 2012.

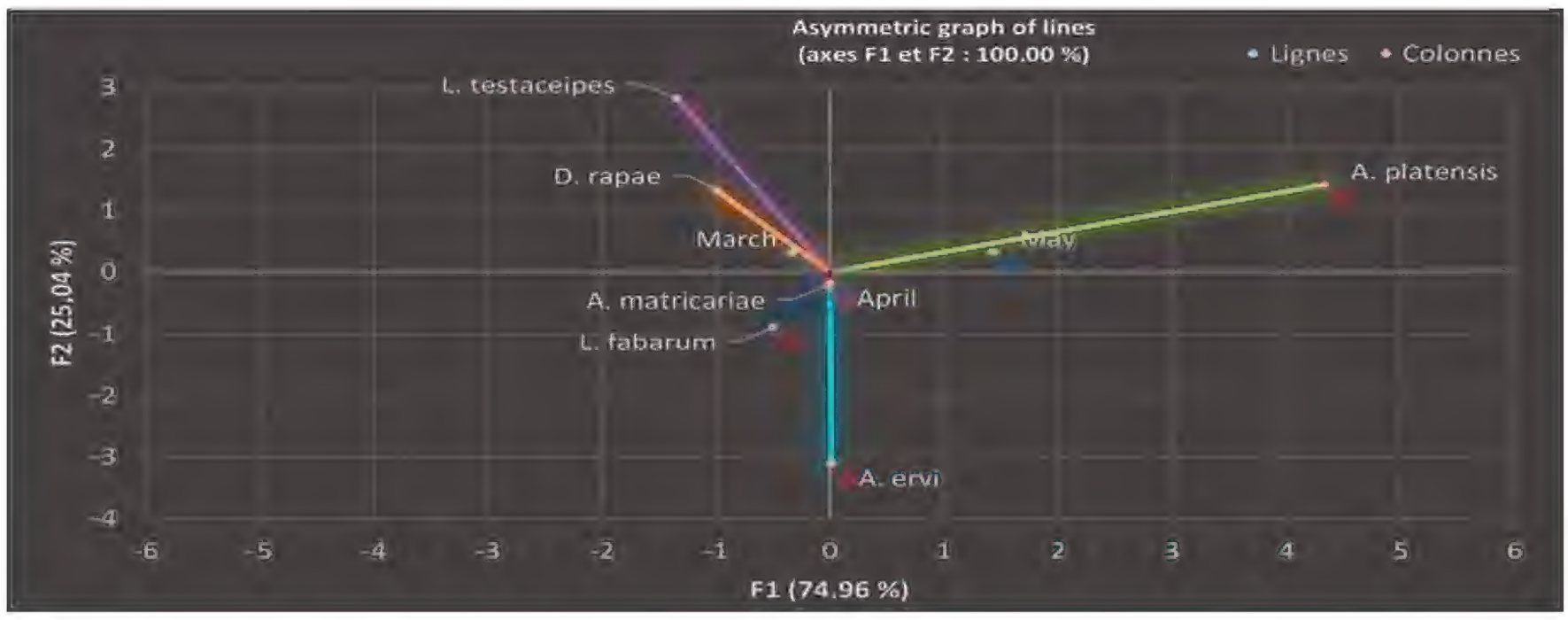

Figure 2. Representation of species inventoried in the A.F.C plan during 2012.

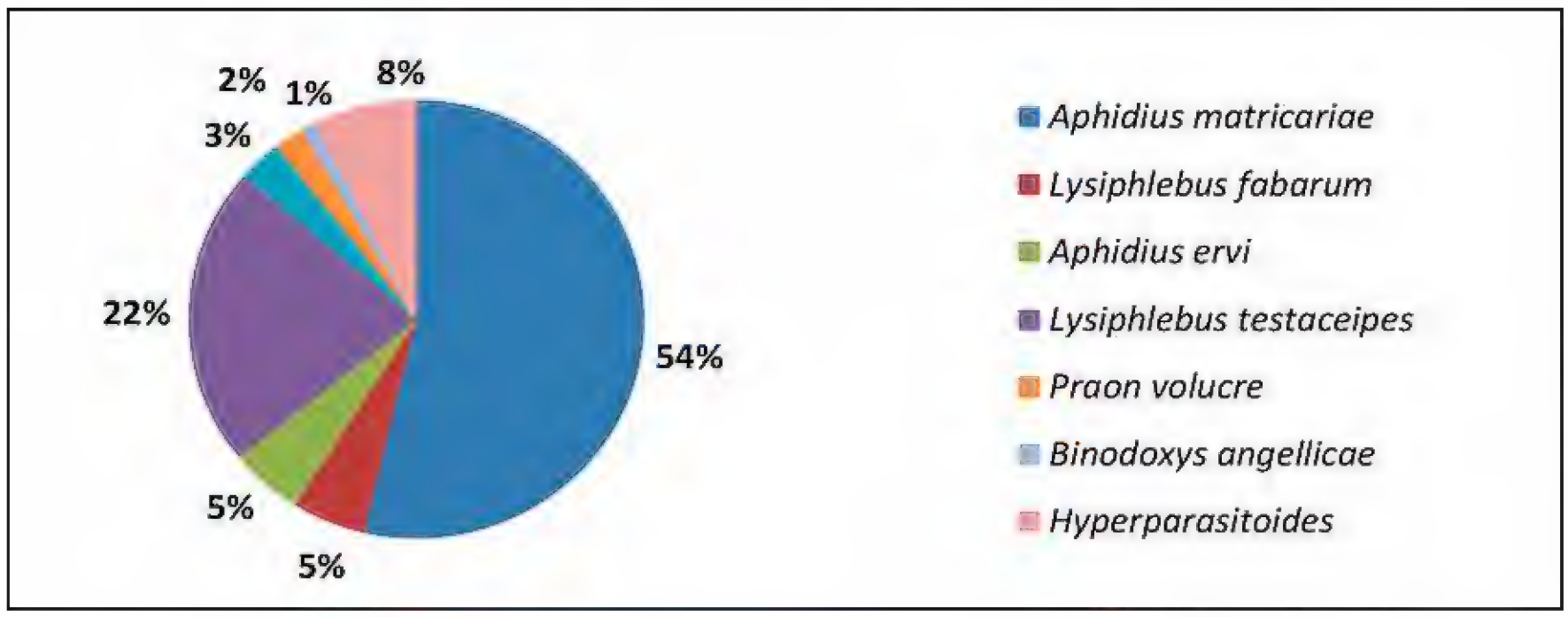

Figure 3. Relative abundance (%) of parasitoids collected during 2013.

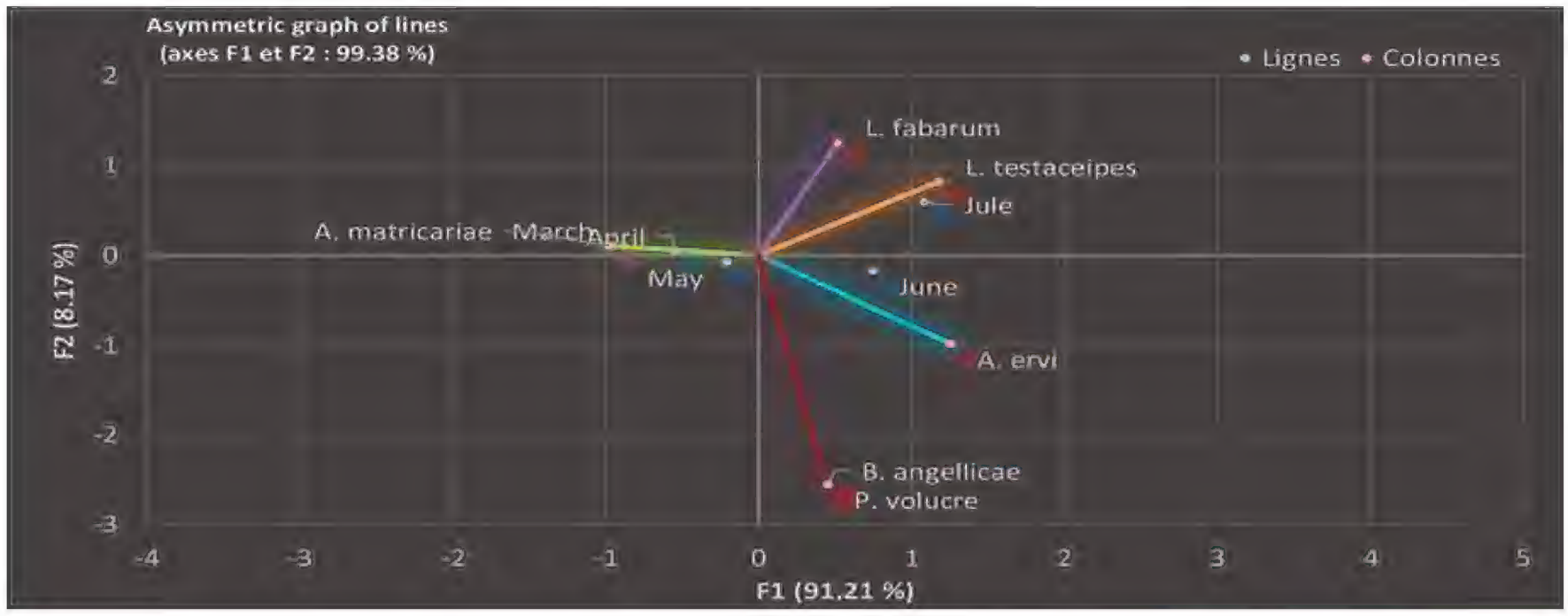

Figure 4. Representation of species inventoried in the A.F.C plan during 2013.

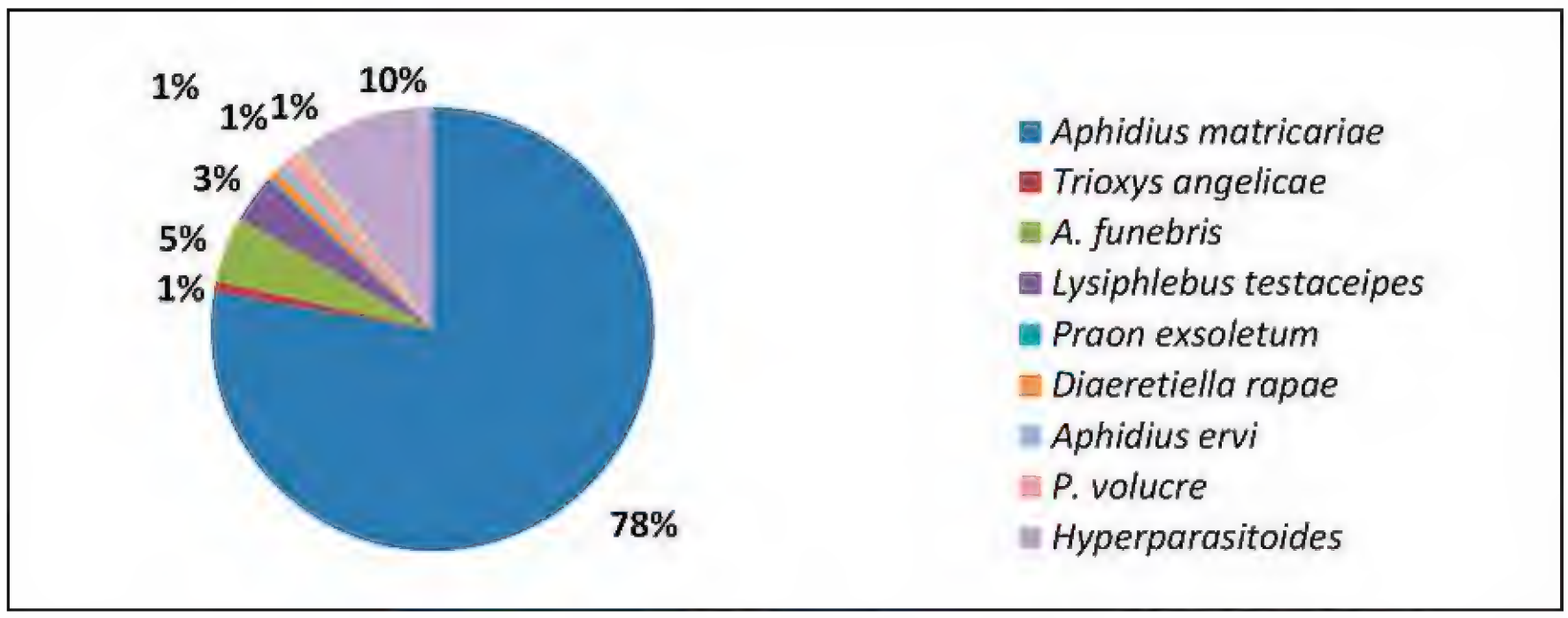

Figure 5. Relative abundance (%) of species of parasitoids identified during the 2014 study period.

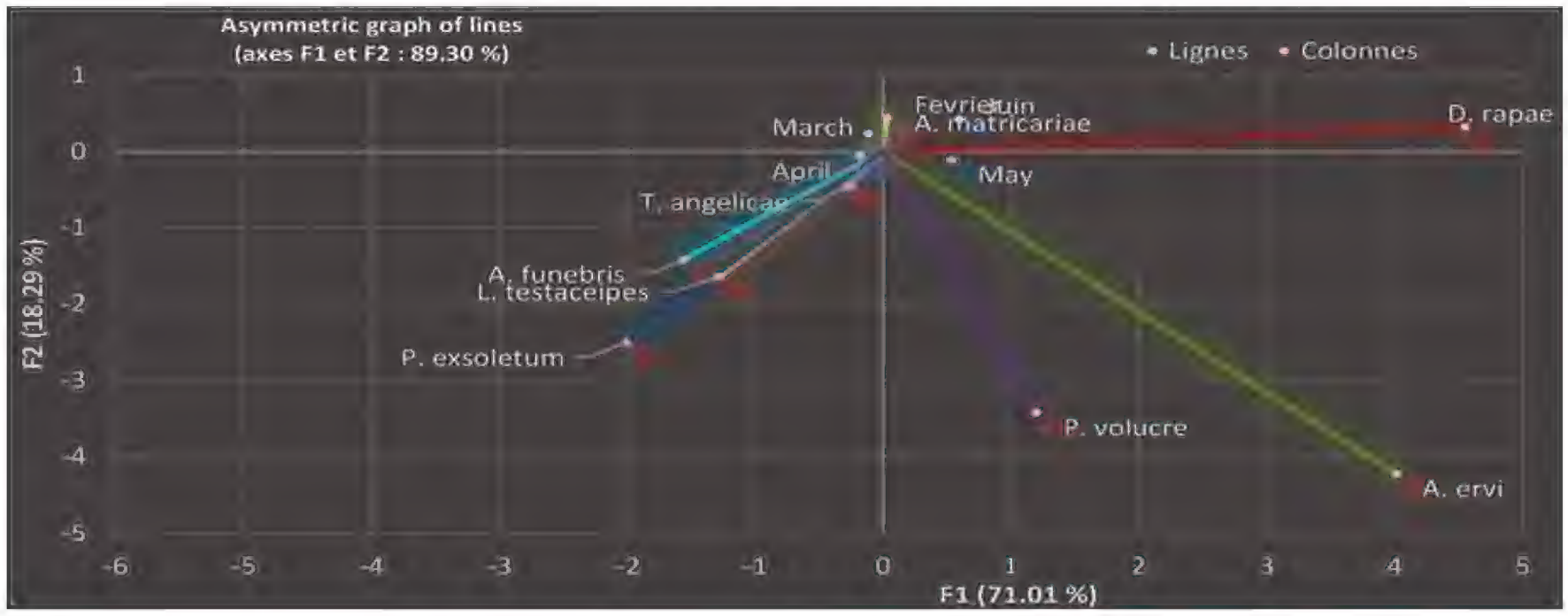

Figure 6. Representation of species inventoried in the A.F.C plan during 2014.

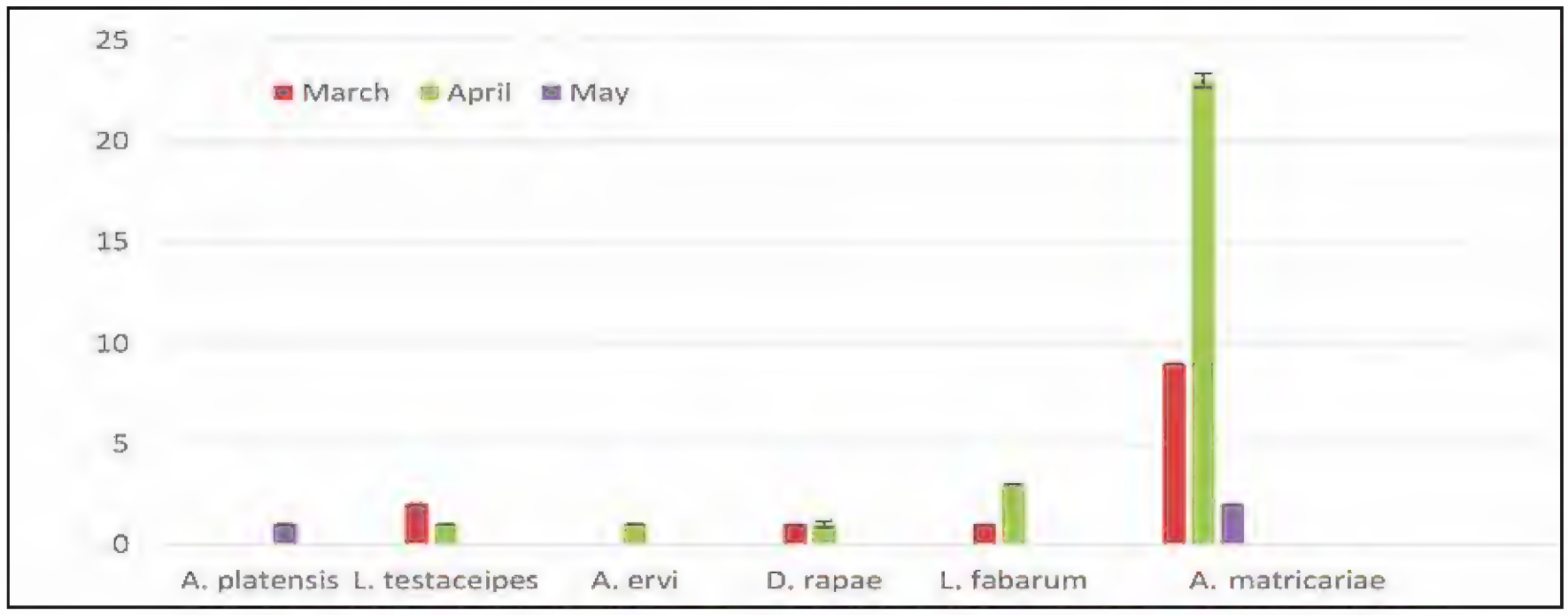

Figure 7. Monthly relative abundance of the various species of parasitoids inventoried during 2012.

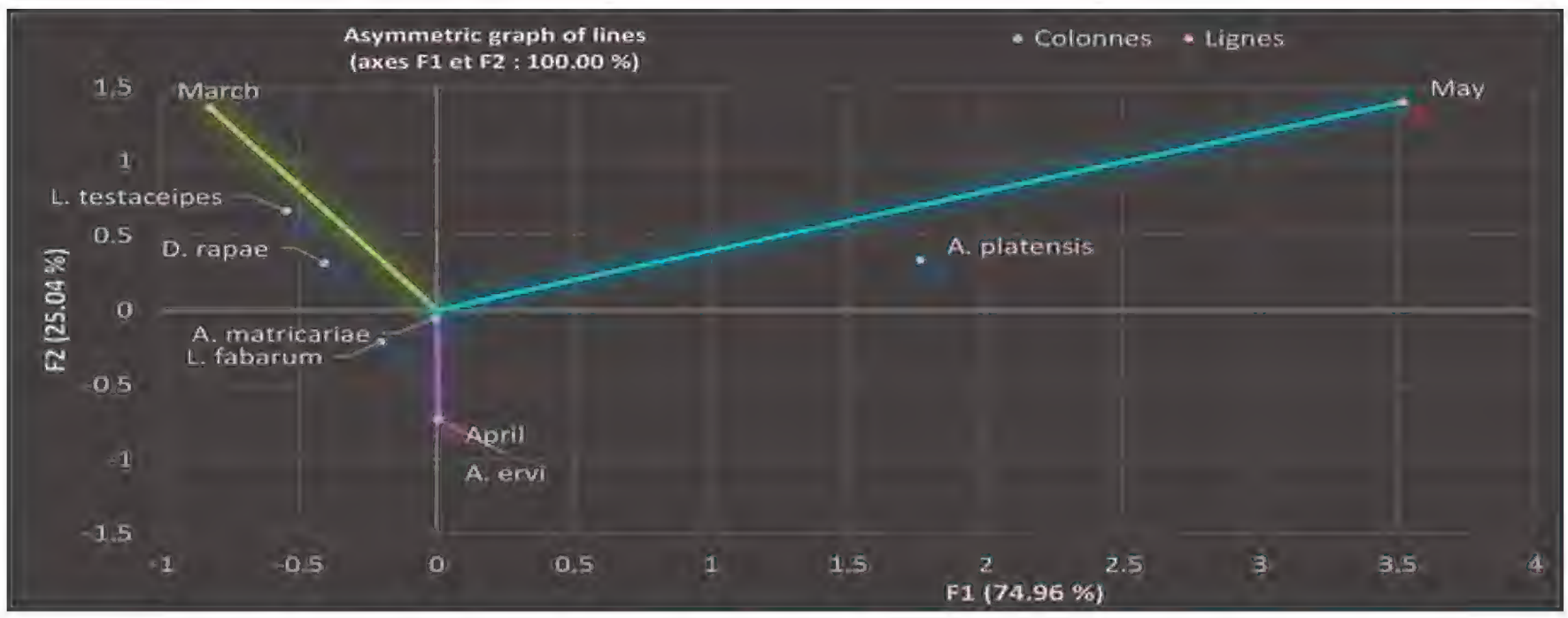

Figure 8. Monthly representation of the abundance of species inventoried in the A.F.C plan during 2012 (effect of months).

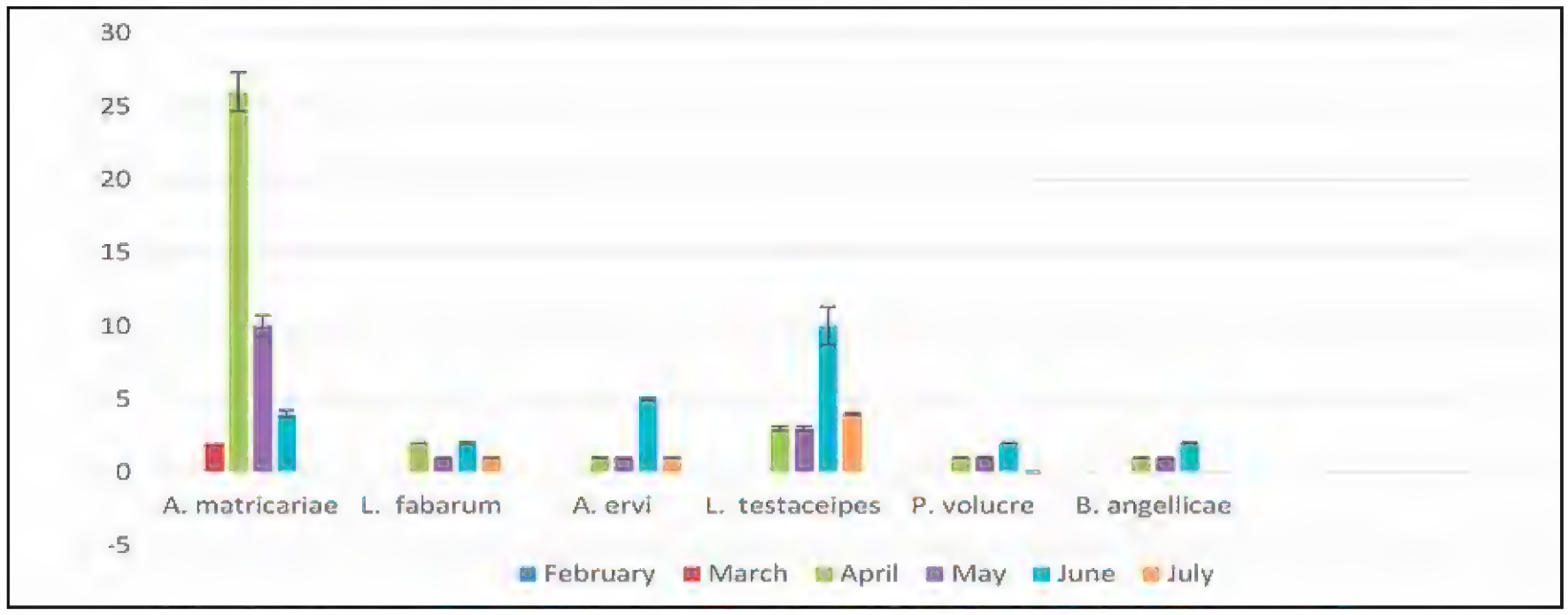

Figure 9. Monthly relative abundance of the various species of parasitoids inventoried during 2013.

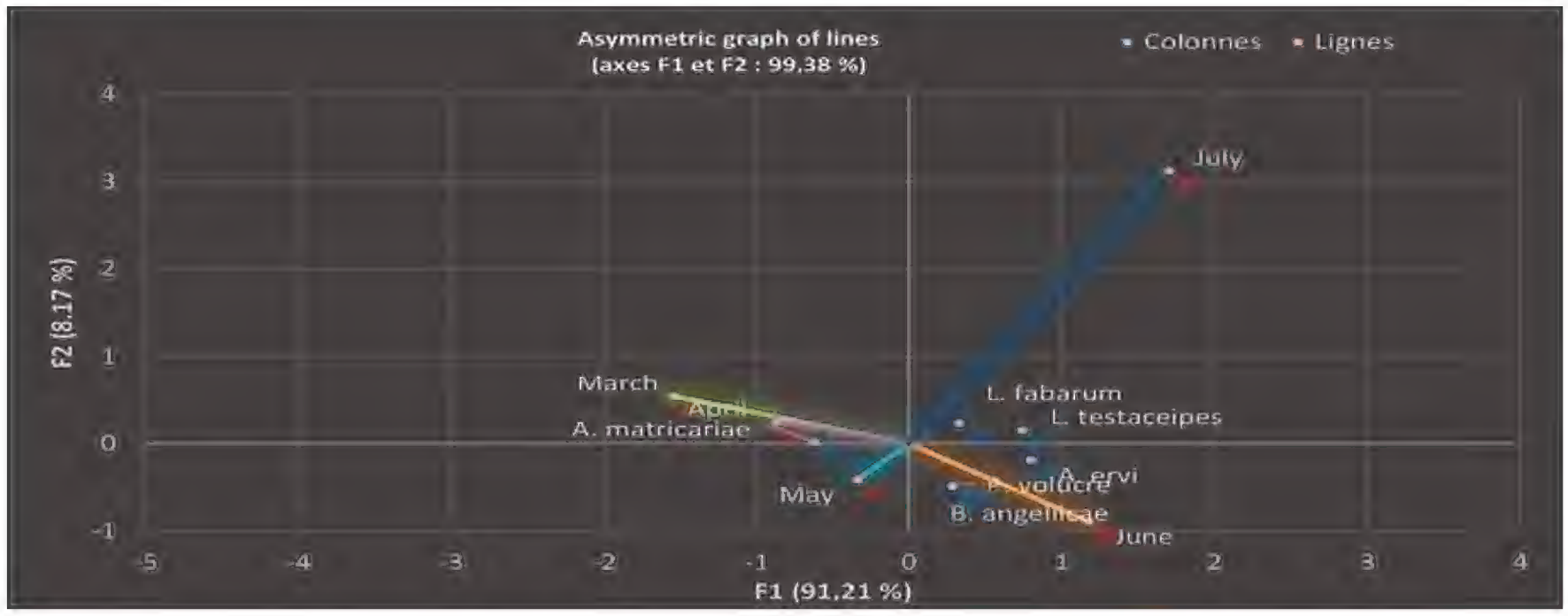

Figure 10. Monthly representation of the abundance of species inventoried in the A.F.C plan during 2013 (effect of the months).

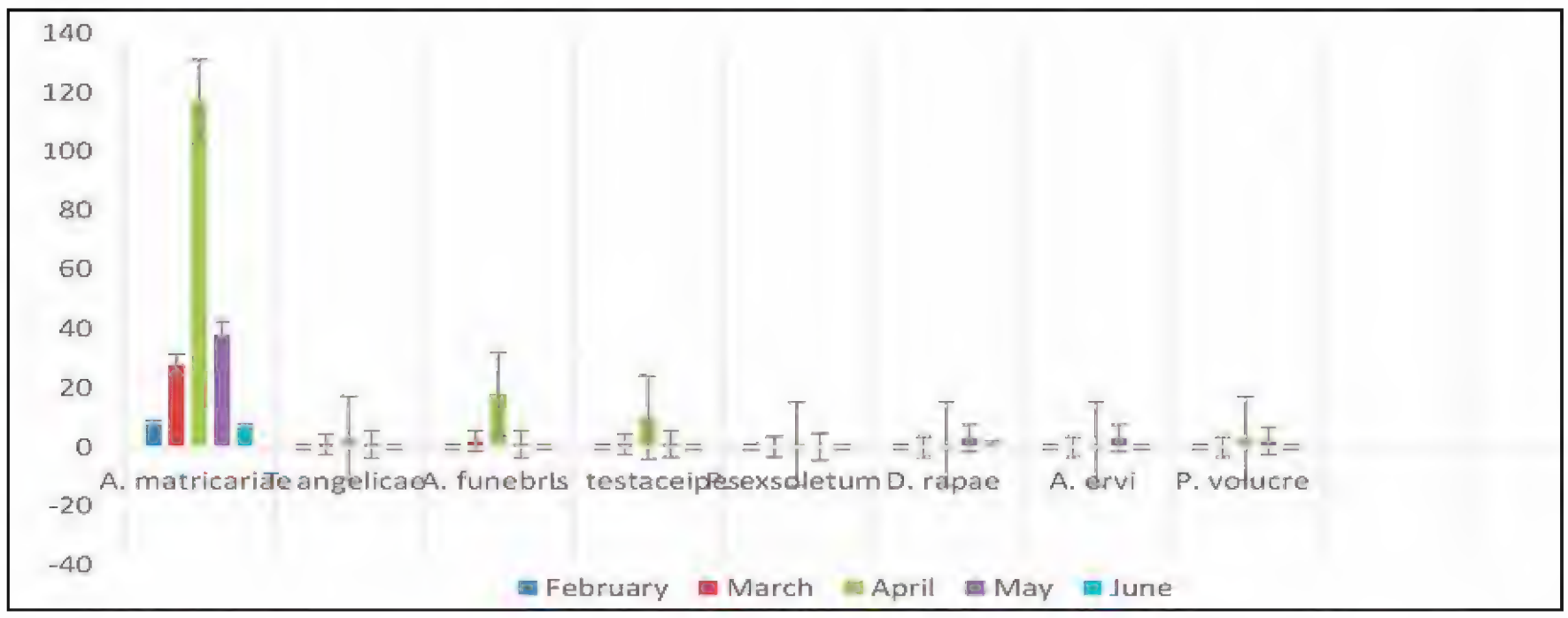

Figure 11. Monthly relative abundance of the various species of parasitoids inventoried during 2014.

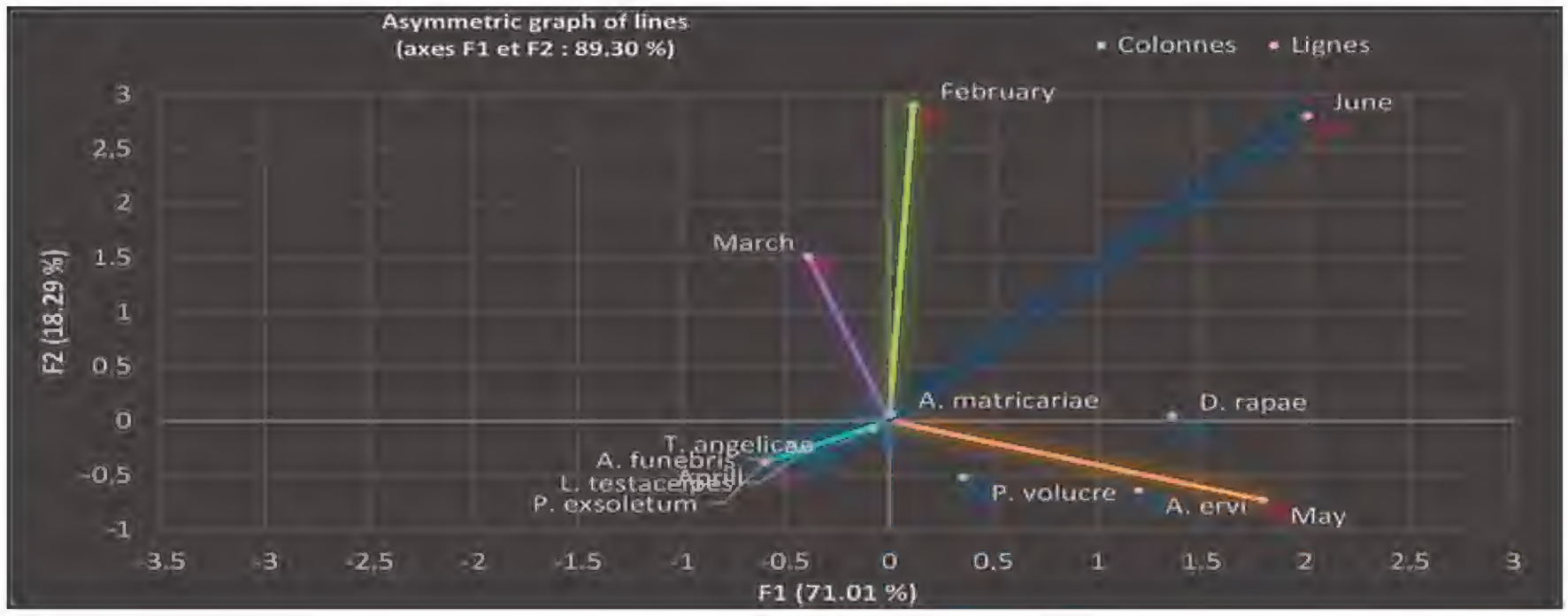

Figure 12. Monthly representation of the abundance of species inventoried in the A.F.C plan during 2014 (effect of the months).

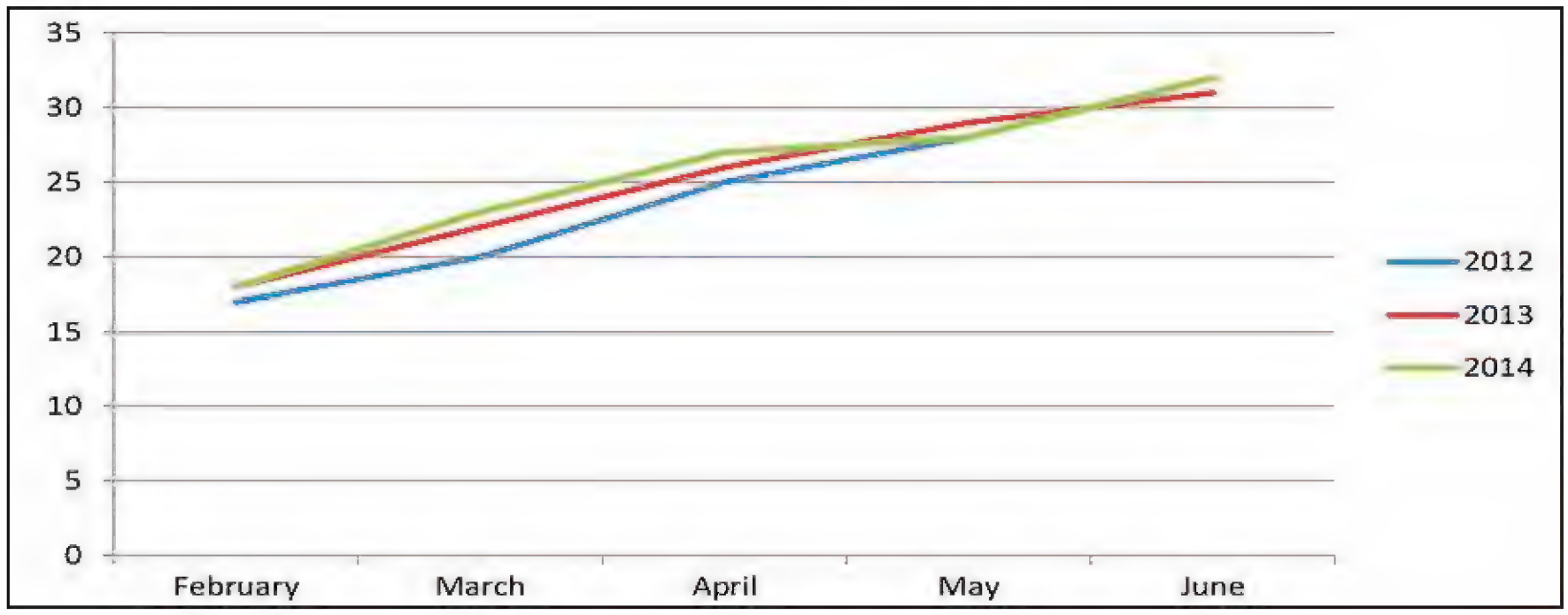

Figure 13. Temperature during the years 2012, 2013 and 2014.

wide distribution throughout the world.

It is important to understand the influence of climatic variations on the functioning of the communities of aphids and their parasitoids. It is also of fundamental importance for the biological control of pest populations (Hance et al., 2007).

Parasitoid performance can be influenced by interaction with other parasitoids, predators and entomopathogens (Rosenheim, 1998; Rashki et al., 2009).

In addition, the abundance and efficacy of primary parasitoids is limited by hyperparasitoid intervention (Darsouei et al., 2011).

Traditionally, hyperparasitoids have been designed to have a negative effect on primary parasitoid populations. There are several ways in which hyperparasitoids can influence primary parasitoid populations: directly by mortality, or indirectly by changing the behavior of parasitoids or the aphids (Buitenhuis, 2004).

In the literature, high rates of hyperparasitism have often been reported. In an agro-ecosystem, the mortality of parasitoids due to hyperparasitism can even reach 100% (Höller et al., 1993). Kanuck & Sullivan (1992) showed that female hyperparasitoids have a preference for the mummified aphid.

Finally, the absence of certain parasitoid species in the study region compared to other regions can be explained by the intensification of modern agriculture, notably by the use of fertilizers and pesticides. This has, of course, led to a decrease in the quantitative and qualitative richness of these parasitoids (Hemidi et al., 2013).

CONCLUSIONS

The populations of *A. matricariae* are the most frequent and have gradually increased to become the most dominant species among aphid parasitoids during the last year of the study, with proportions reaching 78%. The relative and monthly abundances showed us a dominance of *A. matricariae* throughout the study area.

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Biodiversity Journal, 2019, 10 (4): 589–592 https://doi.org/10.31396/Biodiv.Jour.2019.10.4.589.592

Saphanus piceus perovici n. ssp. from Pag Island, Croatia (Coleoptera Cerambycidae Spondylidinae Saphanini)

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ABSTRACT *Saphanus piceus perovici* n. ssp. (Coleoptera Cerambycidae Spondylidinae Saphanini) from Pag Island (Croatia) is here described. The new subspecies is related with the subspecies *Saphanus piceus ganglbaueri* Brancsik, 1886 described from Montenegro and known from the central and southern part of former Yugoslavia, Albania, Bulgaria, and Greece. The new subspecies is very interesting also due to the peculiar habitat where it was collected: very dry and warm.

KEY WORDS Coleoptera; Cerambycidae; Spondilidynae; *Saphanus*; new subspecies; Croatia.

Received 19.11.2019; accepted 16.12.2019; published online 30.12.2019

INTRODUCTION

While studying the Cerambycidae collected in various regions of Croatia we found a small series of *Saphanus* Serville, 1834 collected in Pag Island (northern Dalmatia) that, after a deeper study, appears to belong to a new taxon described in this paper. The genus *Saphanus* until now has been composed by two species: *S. piceus* (Laicharting, 1784) described from Austria (Weiherburg, Insbruck) and *S. kadleci* Rapuzzi et Sama, 2014 described from West Turkey (Sakarya) (Rapuzzi & Sama, 2014). After a revision of the group (Sama & Rapuzzi, 1993; Löbl & Smetana, 2010), *S. piceus* was splitted in three subspecies: the nominal form from Central Europe, Italy, France and northern Balkan peninsula, *S. piceus ganglbaueri* Brancsik, 1886 described from Montenegro (Savina) and widespread in central and southern Balkan peninsula to central Greece and *S. piceus bartolonii* Sama et Rapuzzi, 1993 (Greece, Ossa mountain) known from the eastern mountains of continental Greece (Ossa range and Pilion range) only. The new subspecies is close, due to its characteristics, to *S. piceus ganglbaueri*. It is very interesting due to its biotope as well. It is the very first population of *Saphanus* that is known from the Adriatic islands. The habitat where it was collected is very dry and hot, completely different from any habitat where all the species of the tribe Saphanini normally live, except for *Oxypleurus* Mulsant, 1839 (Fig. 1).

RESULTS

Systematics

Ordo COLEOPTERA Linnaeus, 1758
Familia CERAMBYCIDAE Latreille, 1802
Subfamilia SPONDYLIDINAE Serville, 1832
Tribus SAPHANINI Gistel, 1848
Genus *Saphanus* Serville, 1834
Species *piceus* (Laicharting, 1784) Serville, 1832

Saphanus piceus perovici n. sp. (Figs. 2, 3)

Holotypus male. Croatia, Pag Island, Sveti

Petar, north of Košljun, 44,427270°N 15,038829°E, 11.VII.2015, collected at light, T. Koren legit (coll. T. Koren). Paratypes: 1 male and 1 female, same data as holotype (P. Rapuzzi collection and T. Koren collection).

DESCRIPTION OF THE HOLOTYPE. Length 17.5 mm, width 5.5 mm. Color pitchy black, antennae slightly lighter. Head long, deep punctured, with a deep furrow in the middle of the front and between the eyes. The points are big and deep, sometimes fused together. Pubescence made by long erect brownish setae, more concentrated near the eyes and the antennal tubercles. Eyes big, deeply emarginate. Cheeks short. Antennal tubercles prominent, widely separate in the middle. Pronotum as long as wide, constricted toward the base. Lateral sides with a prominent and acute tooth positioned just up to the middle. Pronotum with very regular and deep punctures, with a shining and impunctate line in the middle. Pubescence on pronotum made by semi-erect hairs mainly concentrated close to the lateral sides. Scutellum longer that wide, rounded apically. Very thin punctures with short black recumbent setae. Elytra with parallel sides, rounded apically. The elytral punctation is regular, made by dense, medium-sized punctures with the same density all over the elytra except for the apical fifth where they are lighter and sparser. From each point starts a short, semi-recumbent, dark brown setae. On the disk there are the vestiges of three carina on each elytron that are quite covered by the regular punctation. Legs long and strong. Femora enlarged with a tooth on the inner side of the medium and hind legs. Tibiae arched, strongly in the middle and hind ones. Punctation made by dense points larger and stronger on the tibiae than the femora. The inner side of each tibia shows a sculpture made by many granules that gives a wrinkled appearance. Slender antennae, reaching last fifth of the elytral length. Third segment twice than the second and fourth little shorter than the scape. Segments from the fifth to the tenth with an apical tooth on the outside side, more evident and prominent from the seventh segment to the tenth. Maxillary palps very long, the apical segment axe-shaped.

VARIABILITY. The second known male (length 16.0 mm., width 5.0 mm.) is very similar to the type. The color of the legs is a little lighter and the vestiges of the elytral carina are less visible. The female (Fig. 3), only one female specimen available (length 17.5 mm., width 7.5 mm.), shows the same dimorphism typical of the genus *Saphanus*. The length of antennae is shorter, exceeding the half of the elytral length only with the last segment. The elytral shape is wider, long-oval instead parallel side. The pronotum is more convex and the legs are stouter and shorter.

DISTRIBUTION AND BIOLOGY. *Saphanus piceus perovici* n. ssp. was collected by light traps in a very dry area of Pag Island, Croatia (Fig. 1). All the species of the genus *Saphanus* are normally collected in mountain forests, in cold and wet regions. It is very interesting to note that the vegetation of the surroundings where the new subspecies was collected are made by a vegetation made by overgrown karstic grasslands with *Juniperus* bushes. Accordingly, it is very likely that the larva feed on the roots of *Juniperus* sp., the only large plant found in the area.

ETYMOLOGY. We dedicate this new subspecies to Mag. Franjo Perović, a former curator of the Natural HistoryMuseum in Zagreb (Croatia). He dedicated most of his life to study insects and to collect a rich entomological collection of diverse orders, inspiring many generation of entomologists.

REMARKS. *Saphanus piceus perovici* n. ssp. is close to *S. piceus ganglbaueri* due to the stouter shape of the body, the shorter antennae and legs and the absence of evident carina on elytra. A new characteristic distinguish *S. piceus piceus* and *S. piceus ganglbaueri* and it is the ratio between the second and the third and the ratio between the fourth and fifth antennal segments. In the nominal form the third segment is more than three times longer than the second and in the subspecies *S. piceus ganglbaueri* it is little more than two times longer. The fourth segment is similar in length to the fifth in *S. piceus piceus* and evidently shorter in *S. piceus ganglbaueri*. The new subspecies shows the same combinations of *S. piceus ganglbaueri* but the third and the fourth segments are even shorter. Due to this, it is more similar to *S. piceus bartolonii*. It can be distinguished from all the other subspecies of *S. piceus* due to the very regular sculpture on the pronotum, made by regular points. These points are not so regular in all the other subspecies. The shining line on the middle of pronotum is completely missing (or only very thin

1

2 3

Figure 1. The habitat on Pag Island (Croatia) where *Saphanus piceus perovici* n. ssp. was collected.
Figure 2. *Saphanus piceus perovici* n. ssp., holotypus. Figure 3. *Saphanus piceus perovici* n. ssp., paratypus female.

and short) in *S. piceus ganglabueri*. The vestiges of the elytral carinas are more evident than in *S. piceus ganglabueri*.

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Biodiversity Journal, 2019, 10 (4): 593–600 https://doi.org/10.31396/Biodiv.Jour.2019.10.4.593.600

Influence of total length, sex and seasonal variations on hematological parameters in *Cyprinus carpio* (Linnaeus, 1758) (Pisces Cyprinidae) in Lake Tonga (Algeria)

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ABSTRACT

Hematological parameters are used as an essential tool to assess the health status of fish. This study aims to provide a background on hematology in Lake Tonga's *Cyprinus carpio* (Linnaeus, 1758) (Pisces Cyprinidae) and to demonstrate the impact of seasonal variations, sex and size on hematology. The study was conducted throughout 2018 and involved 120 individuals sampled monthly and randomly. The specimens were weighed and measured. Blood samples were collected to determine hematocrit (Ht), hemoglobin (Hb), red (GR) and white (GB) blood cell count (Lym, Mon, Gra) and erythrocyte constants: mean blood cell volume (VGM), mean blood cell content (TGM), mean corpuscular hemoglobin concentration (CCMH). The results revealed that the majority of the parameters studied did not show significant differences in size classes, and the statistical comparison between the two sexes revealed significant differences in the values of GR, GB, Mon, Mon, Gra, Ht, Hb, TGM and CCMH. On the other hand, all the parameters studied varied significantly over the seasons.

KEY WORDS *Cyprinus carpio*; hematological parameters; Lake Tonga; sex; size.

Received 28.10.2019; accepted 17.12.2019; published online 30.12.2019

INTRODUCTION

Aquaculture in Algeria has become an area of increasing interest to the government and private sector producers, especially breeding activities and the introduction of new fish species.

The common carp *Cyprinus carpio* (Linnaeus, 1758) is a freshwater fish that is common worldwide (Welcomme, 1998). This global distribution of carp is linked to the many introductions around the world to promote aquaculture and sport fishing (Hoffmann, 1995; Copp et al., 2005; Balon, 2006). Fish are closely associated with their environment; physical and chemical changes in the environment are rapid and uncontrollable and can result in measurable physiological changes in fish (Fazio et al., 2013).

Hematological indices are important parameters for assessing the physiological state of fish and for defining the influence of different environmental factors, pollution or stress on their health (Romestand et al., 1983; Adams et al., 1993; Chen et al., 1995; Houston, 1997). Their changes depend on species, age, sex, sexual maturity cycle and health status (Sniezsko, 1960; Summerfeld, 1967; Blaxhall, 1972; Wedemeyer et al., 1983; Golovina & Trombicky, 1989; Zhiteneva et al., 1989; Bielek & Strauss, 1993; Golovina, 1996; Luskova, 1997; Vosyliene, 1999; Hrubec et al., 2001).

Other factors that can significantly affect hematological parameters in teleosts include reproductive cycle, diet, temperature, pH and photoperiod (Sandnes et al., 1988; Kavadias et al., 1996; Svoboda et al., 2001; Guijarro et al., 2003; Kavadias et al., 2003; Bayir, 2005), or stress, pollution, parasitism, size, seasonal variations (Clarks et al., 1979; Barham et al., 1980; Ranzani-Paiva et al., 2004; Camargo et al., 2005; Santos et al., 2009; Onyia et al., 2013; De Souza Neves et al., 2014; Brum et al., 2014; Figueiredo et al., 2014; Fallah et al., 2014).

The aim of this study is to obtain basic information on the haematology of *C. carpio* from Lake Tonga, which is of interest in improving diagnostic and prognostic arguments by regularly monitoring samples from a fish or aquaculture population in order to achieve better control of farming conditions.

MATERIAL AND METHODS

Study area

Lake Tonga is located in the El-Kala National Park in the extreme north-east of Algeria (36°53' N and 08°31' E). It occupies a vast coastal depression of 2600 hectares with a length of 7.5 km and width of 4 km (Fig. 1); it has been classified as a World Heritage Site and a RAMSAR site of international importance since 1983. This endorheic freshwater lake is currently the result of various works carried out over the past century and has become a marsh pond, communicating with the sea through an artificial channel, the Messida (Gehu et al., 1993).

The Lake Tonga catchment area, with a water volume of about 28,000,000 m^3, which is significantly higher during periods of high water, includes two major rivers that flow all year round (Oued El Hout, 14 km long, and Oued El Eurg, 10 km long) and an outlet, which is Oued Messida (Bentouili, 2007).

The study region is subject to a Mediterranean climate characterized by two different seasons: a humid season, marked by heavy rainfall and low temperatures from October to May, and another dry and hot season with high temperatures reaching their maximum in August (Labar, 2004; Mebarki, 2010).

Sampling

120 specimens of common carp, *C. carpio* were regularly caught and sampled randomly and monthly from January to December 2017 using eel traps. The total length (Lt, cm) of each fish was measured.

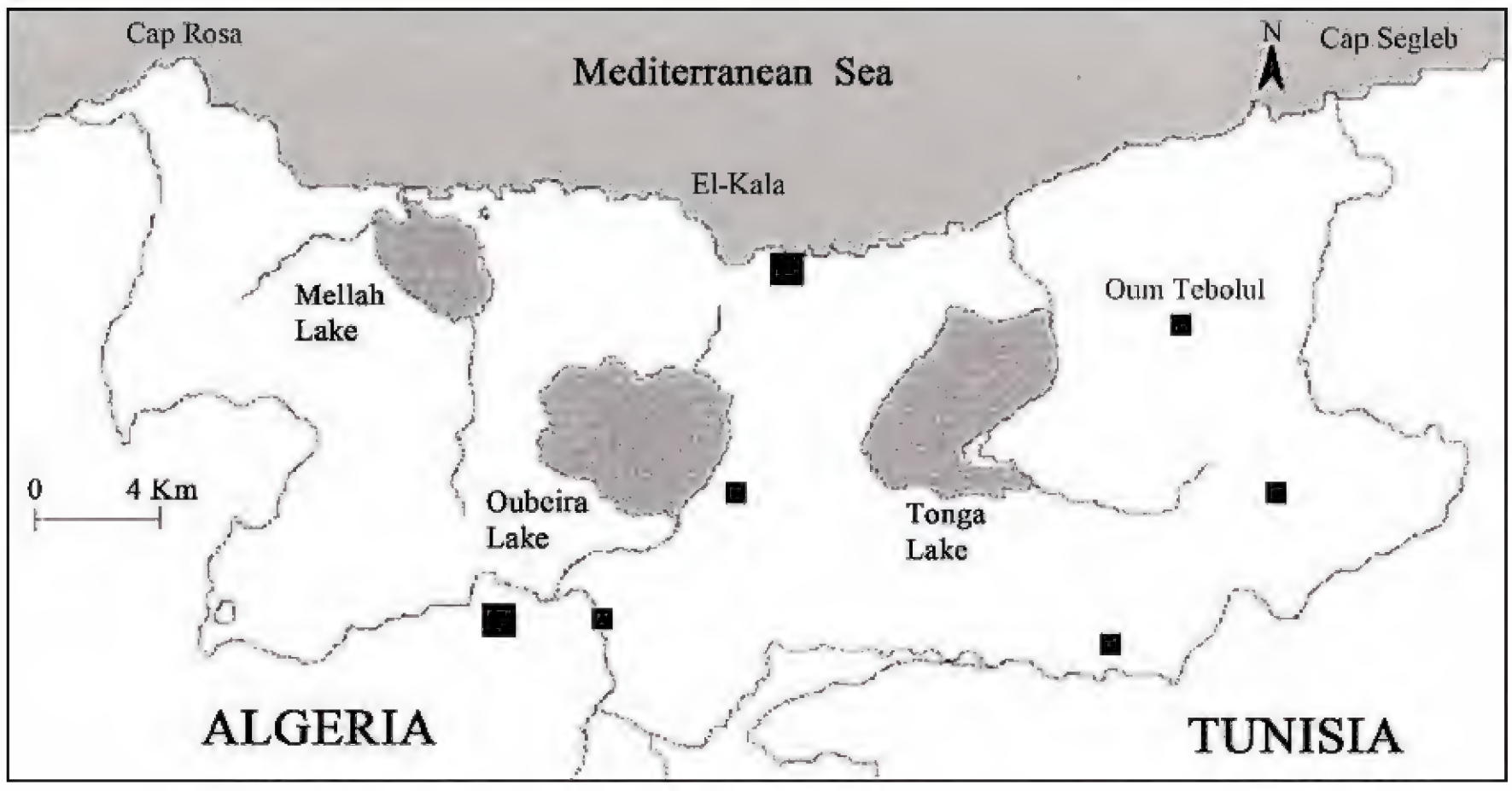

Figure 1. Geographical location of El-Kala National Park (Benyacoub, 1996).

Blood samples were collected immediately on site as soon as the fish were caught by puncture of the caudal vein using a syringe containing a 10% anti-coagulant -EDTA.

Hematological parameters determinations

Hematological parameters were measured by the following traditional analytical techniques: a 540 nm absorbance spectrophotometer using the hemoglobin cyanide procedure for hemoglobin (Hb), by centrifugation of hematocrit capillaries for hematocrit (Ht) and by counting red cells (GR) and white cells (GB) on Thomas cells after dilution in Rees solution (1 g bright cresyl blue, 31.3 g sodium citrate, 10 ml formol 37% and 1000 ml distilled water).

Leukocyte count was performed using stained blood smears with Giemsa / May-Grunwald stain solution (Rosenfeld, 1947); the smears were then examined under an optical microscope (Olympus, Tokyo, Japan) using an immersion oil at a magnification of x100 to obtain the percentage of lymphocytes (Lym), monocytes (Mon) and granulocytes (Gra).

The erythrocyte constants were calculated according to the Wintrobe method (1934) as follows: GMV: mean globular volume (in fl) =Ht (in l/l)/Nb red cells (Tera-/l); GMT: mean globular content (in pg) =Hb (g/l)/Nb red cells (Tera-/l); CCMH: mean corpuscular hemoglobin concentration (in g/) =Hb (g/l)/Ht (l/l).

The data were analyzed by ANOVA for analysis of variance with a significance of 5%, and the means were compared by the Tukey test. The differences were considered significant at $p \leq 0.05$. Minitab statistical software (version 17) was used for all statistical analyses.

RESULTS

The total length of the 120 fish studied ranged from 21.4 cm to 61.4 cm (average 33.73 ± 8.82). Using Sturges' law, the specimens examined were grouped into 8 size classes of 5 cm in amplitude. The statistical comparison between different size classes revealed that the majority of the parameters studied did not show significant differences (Table 1).

The largest fish (56–61cm) had the highest levels of GR, GB, Hb, Mon and CCMH.

Table 2 shows the results of the hematological indices for males and females. The statistical comparison between the two sexes revealed significant differences in the values of GR, GB, Mon, Mon, Gra, Ht, Hb, TGM and CCMH. Higher amounts of Ht, Hb and Lym were reported in males, while higher values of GR, GB, VGM, TGM, CCMH, Mon and Gra were reported in females. Analysis of the hematological parameters studied for *C. carpio* caught in Lake Tonga waters showed that all values varied significantly over the seasons. In contrast, Hb was the only non-significant parameter (Table 3).

The highest values of Mon, Gra, TGM and CCMH were recorded in the spring. In summer, the number of Lym, GR and Hb reach their maximum. In contrast, the highest numbers of GB and GMVs were observed in winter. Hematocrit is the only parameter that reached its peak in autumn.

DISCUSSION

Hematology can be used to study fish respon-

Parameters	Size classes								P-value
	21-26 n=31	26-31 n=14	31-36 n=39	36-41 n=10	41-46 n=14	46-51 n=6	51-56 n=3	56-61 n=3	
GR (×106 µl)	1.24±0.24	1.36±0.24	1.27±0.34	1.38±0.37	1.43±0.26	1.38±0.18	1.22±0.08	1.65±0.32	0.177
GB (×103 µl)	64.5±8.75	61.18±9.76	62.54±9.39	61.77±8.83	57.41±7.07	60.78±8.14	61.6±12.44	72.3±5.23	0.184
Ht (%)	19.3±4.53	20.69±5.11	18.2±6.14	19.49±6.92	23.66±5.09	22.67±5.48	19.8±4.4	19.47±3.73	0.096
Hb (gr/dl)	10.5±1.38	10.95±0.92	10.91±1.18	11.88±1.39	10.07±2.05	11.06±0.89	9.8±1.37	13.16±1.45	0.003**
VGM (fl)	157.89±29.7	151.72±24.69	142.6±23.62	141.31±28.71	166.27±23.26	162.18±20.74	161.5±32.5	118.33±1.29	0.012*
TGM (pg)	85.93±10.89	82.23±14.03	93.36±30.49	91.95±27.07	70.11±6.14	80.45±8.26	79.9±10.41	80.63±7.94	0.045*
CCMH(gr/dl)	55.89±9.32	55.6±12.57	67.94±26.22	68.06±23.43	43.25±8.87	50.97±12.02	52.1±18.9	68.4±6.26	0.001**
Lym (%)	86.2±7.68	87.21±6.45	87.92±5.13	86.73±5.25	88.21±3.16	84.63±4.05	86.2±4.84	84.8±2.99	0.816
Mon (%)	7.98±2.57	7.69±2.7	7.6±2.46	8.25±2.47	7.36±1.95	7.88±1.26	7.37±3.15	10.3±1.24	0.715
Gra (%)	5.78±5.38	5.16±3.93	4.47±2.85	5.02±3.04	4.42±1.82	7.48±2.95	6.43±2.01	4.9±1.74	0.61

Table 1. Comparison of hematological indices in *Cyprinus carpio* from Lake Tonga as a function of total length.
P> 0.05 = Not Significant; P < 0.05 = Significant (*); P < 0.01 = Highly Significant (**).

Parameters	Males n=50	Females n=70	P. Value
GR (×106 µl)	1.51 ± 0.23	1.81 ± 0.26	<0.001**
GB (×103 µl)	57.98 ± 6.93	65.48 ± 9.14	<0.001**
Ht (%)	22.54 ± 5.24	17.87 ± 5.06	<0.001**
Hb (gr/dl)	11.13 ± 1.75	10.61 ± 1.14	0.054
VGM (fl)	150.56 ± 29.55	151.51 ± 25.31	0.85
TGM (pg)	74.21 ± 9.01	94.44 ± 24.04	<0.001**
CCMH (gr/dl)	51.84 ± 14.15	64.6 ± 21.66	<0.001**
Lym (%)	90.29 ± 2.93	84.27 ± 6.18	<0.001**
Mon (%)	6.45 ± 1.59	8.78 ± 2.42	<0.001**
Gra (%)	3.26 ± 1.79	6.48 ± 4.13	<0.001**

Table 2. Comparison of hematological indices between males and females of *Cyprinus carpio* from Lake Tonga. P > 0.05 = Not Significant; P < 0.05 = Significant (*); P < 0.01 = Highly Significant (**).

siveness to different environmental conditions, which allows a better understanding of their physiology and health status and to develop the most optimal environments for aquaculture. Hematology can also be used in the diagnosis and treatment of diseases (Ranzani-Paiva et al., 2013).

In recent years, the study of fish haematological parameters has received increasing attention and has become a key issue for aquaculture. However, several factors are likely to modify the blood parameters of a fish (size, sex and seasonal fluctuations, etc.).

The findings presented in this study on the effect of seasonal variations, sex and size on hematological parameters in *C. carpio* from Lake Tonga showed that many hematological indices differed significantly with respect to sex and seasonal variations, but were not significant with respect to total length.

The results obtained after the total length effect study show that the rates of GR, GB, Hb, Mon and CCMH were higher in the longest fish. This is consistent with the results of Jawad et al. (2004). The authors found that GR and Hb values increased with increasing fish size. Poston (1966) and Anthony et al. (2010) also observed that the number of GR and Hb tends to increase with the length and age of the fish. As a result, rapid increases in body weight, as well as an increase in blood volume, are accompanied by adequate erythropoiesis (Svetina et al., 2002).

In addition, it may also be the result of changes in plasma or erythrocyte volume (Sandstrom, 1989). However, Chaudhuri et al. (1986) suggested that this relationship may be due to the higher metabolic rate in large fish compared to smaller.

Furthermore, Ranzani-Paiva (1995), Svetina et al. (2002) and Baghizadeh & Khara (2015) suggested that the CCMH increased with the age of the *C. carpio* carp, which would be due to adaptation strategies adopted at different life stages.

However, no significant change in hematocrit level relative to total length was observed, despite the general trend observed in the relationship between blood hematocrit and body length of *C. carpio*: the longer the fish, the higher the hematocrit, as reported by Murachi (1959), Svetina et al. (2002) and Hrubec et al. (2001) for mature hybrid tilapias, *Oreochromis* spp. and Orun & Erdeml (2002), in the case of the long spine scraper, *Capoeta trutta* (Heckel, 1843).

GBs are defense cells of the body. According to Douglass and Jane (2010), their levels influence immune responses and the animal's ability to fight infection. White blood cell counts are frequently used as an indicator of health status for fish, as for other vertebrates. These cells are key components of the innate immune defense and participate in the regulation of immunological function in the body (Ballarin et al., 2004). The GB values do not show any significant difference between the size classes. On the other hand, differences in hematological parameters by sex of fish have already been proven (Gabriel et al., 2004; Akinrotimi et al., 2007).

Many studies have shown that males show the highest values in almost all hematological indices; these high values are attributed to greater physiological activity in males (Cech & Wohlschlag, 1981; Orun et al., 2003). Moreover, higher metabolic activity in males may lead to differences in blood cell components (Collazos et al., 1998). Parma & Croux (1994) demonstrated that the variation in hematological indices between the two sexes could be due to differential oxygen demand.

The findings of Fourie & Hattingh (1976) and Baghizadeh & Khara (2015) for *C. carpio* and those of Jawad et al. (2004) for *Tenualosa ilisha* F. Hamilton, 1822 are in agreement with the results of this study, which showed that Hb, Ht and Lym were higher in males than in females.

Whereas, the results of Orun et al. (2003) as well as Orun & Erdeml (2002) for cyprinids were quite the opposite of the results of this study, showing that the number of red and white blood cells in males was lower than in females. Differences in leukocyte count could be attributed in particular to

Parameters	Winter n=30	Spring n=30	Summer n=30	Autumn n=30	P. Value
RG (×106 µl)	1.21±0.18	1.09±0.33	1.53±0.20	1.43±0.23	<0.001**
GB (×103 µl)	67.54±7.31	66.86±9.7	54.07±4.97	60.94±6.51	<0.001**
Ht (%)	19.8±3.64	15.46±4.96	21.95±6.12	22.05±4.96	<0.001**
Hb (gr/dl)	10.71±1.08	11.08±1.25	10.63±1.76	10.89±1.61	0.641
VGM (fl)	163.53±18.8	142.72±23.19	143.13±31.27	155.08±28.5	0.005**
TGM (pg)	89.27±9.83	108.72±27.86	69.31±6.53	76.74±9.33	<0.001**
CCMH	55.4±9.25	8.54±24.33	51.58±15.38	51.61±13.49	<0.001**
(gr/dl)	84.86±5.7	83.05±5.72	91.98±2.26	88.28±4.23	<0.001**
Lym (%)	8.42±2.06	9.78±2.09	5.81±1.36	7.22±2.09	<0.001**
Mon (%)	6.71±4.16	7.15±3.91	2.20±1.06	4.48±2.58	<0.001**
Gran (%)					

Table 3. Seasonal comparison of hematological indices of *Cyprinus carpio* from Lake Tonga.
(P > 0.05 = Not Significant; P < 0.05 = Significant (*); P < 0.01 = Highly Significant (**).)

stress, age, maturity, gender, pathogens (biotic factors) and/or water temperature, pH, dissolved oxygen content (abiotic factors) (Pavlidis et al., 2007). As a result, all these environmental variations make interpretation difficult.

The majority of hematological parameters were higher in months of high temperature to meet the considerable energy demand of the fish. They had significantly lower values in months of low temperature due to the body's high metabolic rate due to high body temperature and reproductive activities. These results were not in agreement with the work of Joshi (1989), Orun et al. (2003), Adebayo et al. (2007), Khadjeh et al. (2010) and Kohanestani et al. (2013). As a result, it is recommended that further hematological studies be conducted on the same fish species to obtain further results.

CONCLUSIONS

This study aimed to provide information on the hematology of *C. carpio* from Lake Tonga (El Kala-Algeria) based on seasonal changes, sex and size. The study was conducted throughout 2018 and involved 120 individuals sampled monthly and randomly.

Seasonal fluctuations were found to have a significant effect on all parameters studied, with the exception of Hb. Sex had a significant effect on the values of GR, GB, Mon, Gra, Ht, Hb, TGM and CCMH. On the other hand, significant differences in the comparison of total length were observed only in the values Hb, GMV, GMT and CCMH. As a result, these results will serve as preliminary data for further studies.

Therefore, it is recommended that further and more detailed research be carried out to determine the effects of the three factors mentioned on hematological parameters in fish, due to the increasing importance given to fish farming and the increased awareness of pollution of aquatic ecosystems.

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Biodiversity Journal, 2019, 10 (4): 601–607 https://doi.org/10.31396/Biodiv.Jour.2019.10.4.601.607

New ant species of *Myrmicaria* Saunders, 1842 (Hymenoptera Formicidae) from Senegal, a second species with subpetiolar process in this genus

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ABSTRACT

The description of *Myrmicaria morettoi* n. sp. (Hymenoptera Formicidae), new ant species from Senegal is given. This species presents an unusual ring-like subpetiolar process as *M. salambo* Wheeler, 1922, the only species in the genus *Myrmicaria* Saunders, 1842 sharing the armed petiole with the taxon newly described here. A first report of *M. salambo* from Ivory Coast is also given.

KEY WORDS

Ants; Formicidae; Myrmicinae; *Myrmicaria*; new species; Senegal.

Received 02.10.2019; accepted 18.12.2019; published online 30.12.2019

INTRODUCTION

In the recent revision of the subfamily Myrmicinae (Ward et al., 2015), important taxonomic changes have been introduced and the most remarkable one was the reduction in number of tribes from twenty-five to only six. In this new context, the tribe Solenopsidini Forel, 1893 sensu novo also includes *Myrmicaria* Saunders, 1842, a genus with about 70 ant species, diffused from Africa to South East Asia, all characterized by antennae with only seven segments (scape included).

Myrmicaria has always been a neglected genus. For example, regarding the Afrotropical area only, just one revision was published (Santschi, 1925), a study that needs updates now; moreover of the about 30 African species, none has been described recently.

In my personal collection (ASPC), I keep a series of ant specimens belonging to an undetermined *Myrmicaria* from Senegal. In many of these specimens, the petiolar peduncle shows ventrally two extraordinarily developed teeth, often joined apically to form a great ring (Fig. 3). The presence of a subpetiolar process is exceptional in the genus, and so far known only in the African *M. salambo* Wheeler, 1922. This last species slightly differs from my specimens, in the subpetiolar teeth less developed and (apparently) never joined apically, moreover the body is ochre in colour and the sculpture appears more areolate-reticulate, and the costumes are arboreal instead of terrestrial.

For all these reasons, I considered my Senegal's specimens as belonging to a new species that I describe here.

MATERIAL AND METHODS

The materials studied are deposited in the following private and public collections: ASPC (Antonio Scupola, personal collection, Italy), MCZ (Museum of comparative Zoology, Harvard University, USA); MSNV (Natural History Museum, Verona, Italy), MSNG (Natural History Museum, Genoa, Italy), MSNM (Natural History Museum, Milan, Italy). The measurements (min and max) ex-

pressed in mm are taken by an ocular reticule mounted on Leica MB3 stereomicroscope at max 60X magnification. For brevity, in the text the following acronyms are used:

CL (cephalic length – the length of head, excluding the mandibles, measured from the midpoint of the anterior clypeal margin to the mid-point of the posterior margin in full-face view);

CW (cephalic width – maximum head width, measured immediately behind the eyes, with the head in full-face view);

FLD (frontal lobes distance – the maximum distance between the frontal lobes with the head in full-face view);

GL (gaster length – approximatively total length of gaster measured in lateral view);

MH (mesosoma width – the maximum height of the mesosoma, measured in lateral view from the dorsum of mesonotum to inferior edge of the mesopleuron);

ML (mesosoma length – the diagonal length of the mesosoma, measured in lateral view, from the angle at which the pronotum meets the cervix to the posterior basal angle of the metapleuron);

PH (petiole height – the maximum height of the petiole, measured in lateral view from the dorsum of petiolar node to the sternite (subpetiolar process excluded);

PL (petiole length – the length of the petiole, measured in lateral view from the anterior to posterior margin of the petiolar node);

PPH (postpetiole height – the maximum height of the postpetiole, measured in lateral view from the dorsum of postpetiolar node to the sternite);

PPL (postpetiole length – the length of the postpetiole, measured in lateral view, from the anterior to posterior margin of the postpetiolar node);

PPSD (pronotal-propodeal spine distance – the distance from the apex of the pronotal antero-lateral tooth to apex of propodeal spine in lateral view);

PSD (propodeal spines distance – the distance between the apex of the propodeal spines, with the propodeum in dorsal view);

SL (scape length – the maximum straight-line length of the scape, without the basal condyle);

SPSD (spiraculo – propodeal spine distance -the distance from the propodeal spiracle to the apex of the propodeal spine);

TL (total length – the approximate total length of body formed by the sum of CL+ML+PL+PPL+GL).

Indices. CI (cephalic index – CW/CLx100); CS (cephalic size – CW+CL/2); SI (scapus index – CW/SLx100).

RESULTS

Systematics

Ordo HYMENOPTERA Linnaeus, 1758
Familia FORMICIDAE Latreille, 1809
Subfamilia MYRMICINAE Lepeletier de Saint-Fargeau, 1835
tribus SOLENOPSIDINI Forel, 1893
genus *Myrmicaria* Saunders, 1842

Myrmicaria morettoi n. sp. (Figs. 1, 3, 4, 8)

EXAMINED MATERIAL. Holotypus (worker) (MSNV) and 13 Paratypes (workers) (ASPC, MSNG, MSNM): Senegal, Tambacounda, PN du Niokolo Koba, Assirik, loc. Ancien Poste du Mont Assirik, 144 m, 12°53'19"N 12°43'10"O, 21–25.VII.2007, leg. Ph. Moretto & F. Génier.

DESCRIPTION OF HOLOTYPE (worker). Colour: in dorsal view head, mesosoma and nodes of petiole and postpetiole black in colour. In dorsal view, first gastral tergite bicoloured (base and central part brownish-ochre, posterior brownish-black), remaining gastral segments brownish-black. In lateral view, head, mesosoma, petiolar peduncle ochre and nodes of petiole and postpetiole brownish-ochre. Mandibles ochre with masticatory margin brownish-black. Antennae brownish-black with apex of the last funicular segment indistinctly paler. Femora brown, tibiae brownish-ochre, tarsi much paler. Prosoma: in full-face view head sub-quadrate with sides convergent from the compound eyes to peristoma; posterior margin of head flat or weakly convex; maximum width of head before the anterior margin of compound eyes; the latter strongly protruding and placed near the corners of the posterior margin of head. Surface of head smooth and shiny with spaced longitudinal and sinuous carinae. A contiguous sinuous line of anastomosis on the front, in the vertex longitudinal carinae joined them to form a longer areolate sculpture. A median longitudinal and regular well-raised carina is present from frontal triangle to posterior margin of head; before the frontal triangle, this carina shortly enlarged with its

internal space micro rugged. On clypeus, central carina present but less evident. Frons with 5–6 longitudinal carinae on each side of the median line. Antennal fossae deep with 3–4 circular carinae that surround the antennal sockets. In lateral view superior part of malar area with 4–5 longitudinal regular carinae, without anastomosis, the carinae reaching the compound eye and after the eye, they turn around and then come back to peristome. Clypeus with the raised median carina plus two lateral carinae placed in front the frontal lobes, also in the space between the median and the two lateral carinae are present 2–3 short basal carinulae. Mandibles triangular with 5–6 teeth, surface well striated with sparse subdecumbent setae. Antenna of 7 segments (scape, pedicel and five funicular segments). Second funicular segment longer than the first and the third together. Antennal club three-segmented with last segment much elongated. Scape smooth and shiny, weakly striated at the apex only. Mesosoma: surface smooth and very shiny. In dorsal view pronotum and mesonotum with a well-raised median regular carina and 4–5 weakly sinuous longitudinal carinae placed to each sides of the median carina. Space between median and first lateral carina very large and without anastomosis (except in the narrow space near the pronotal anterior margin). All longitudinal pronotal carinae in proximity of the anterior margin of pronotum merged by transverse, short anastomosis. Pronotum with anterolateral inferior tooth very prominent. In lateral view, pronotum with carinae and surface smooth, mesopleurae with regular carinulae but surface rugose, metapleurae with carinulae but surface smooth. In lateral view, posterior part of mesonotum vertical and dorsally lobed and edged. In posterior view, vertical slope of mesonotum without sculpture except the median carina and the margins. In dorsal view, propodeum narrow and concave, lacking median carina and never with punctures or anastomosis, surface of the concavity very smooth and shiny. Propodeal spines elongated. Dorsum of propodeal spines sulcate. Slope of propodeum shorter that dorsal side. In lateral view propodeum with carinae. Propodeal spiracle circular and very prominent. Femora smooth and shiny, weakly striated at the apex only (in lateral view), raised granules at base of setae are also present. Metasoma: petiolar peduncle long, with ventral part armed with a subpetiolar process formed by two long translucent teeth curved at apex and joint apically together to form a ring. A linear translucent lamella is present along the ventral part of petiole. Postpetiole lacking anterior ventral process, therefore anterior ventral margin obtuse never prominent. In dorsal view dorsum of nodes of the petiole and postpetiole narrow laterally. In lateral view anterior and posterior sides of petiolar node weakly convergent to dorsum. In dorsal view, petiolar node with 4–6 spaced longitudinal carinae, postpetiolar node with 4–6 longitudinal carinae very close and joined with circular and regular pattern in the posterior slope. Base of gaster micro rugged and sub-opaque, remaining surface of gaster smooth and shiny. Pilosity: erect and sub-

Figure 1. *Myrmicaria morettoi* n. sp., holotypus.

Figure 2. *Myrmicaria salambo*, habitus of specimen from Mbanto (Boundiali, Ivory Coast).

erect setae only. Pubescence absent. All setae very long, stout and black in colour. Scape dorsally with long erect setae and shorter sub-erect setae, ventrally with short sub-erect setae only. In general, on the body setae always placed on carinae, while in the appendices each setae placed on a granule.

Holotypus measurements: CL: 1.558; CW: 1.517; SL: 1.599; FLD: 0.779; ML: 1.763; MH: 1.353; PPSD: 1.886; SPSD: 0.82; PSD: 0.451; PL: 0.943; PH: 0.533; PPL: 0.451; PPH: 0.574 ; GL: 2.01; CS: 1.537; CI: 97.368; SI: 94.87; TL: 6.725.

VARIABILITY. Paratypes: subpetiolar process may vary in the development of the teeth as follow: teeth joined apically (ring-like) as the holotype in 5 workers; teeth aren't joint apically in 3 workers; teeth absent but a translucent narrow linear lamella along the venter of petiole it's well visible in 5 workers. Measurements: see Table 1.

ETYMOLOGY. The new species is dedicated to the French entomologist Philippe Moretto (valued specialist of scarabaeids Ontophagini), collector of the type specimens.

REMARKS. *Myrmicaria morettoi* n. sp. is characterized by two longer teeth placed in the ventral part of petiolar peduncle, often bent and joined apically (Figs. 1, 3) to form a great ring-like process. A linear translucent linear lamella is also present. Until now, in the genus *Myrmicaria*, only *M. salambo* Wheeler, 1922, from Congo has a subpetiolar process (Fig. 5). Both species are distinguishable by the following characters:

Myrmicaria morettoi n. sp.: size TL 6–7.8 mm. Brownish black in colour with petiolar peduncle and gaster castaneous or brownish-ocre. Clypeus with median carina absent or faintly traced. Femora shiny and weakly striated only at apex. Petiole with subpetiolar process formed by two long teeth joined apically (ring-like) (Fig. 3) or separate apically or totally absent in some cases (Fig. 4). Venter of postpetiole anteriorly obtuse (never angled or pointed). Pronotum and mesonotum with median raised carina, remaining carinae linear without anastomosis (except those on the anterior margin of pronotum). Dorsum of propodeum smooth and lacking longitudinal carinae or anastomosis. Nodes of peduncle carinated. Postpetiolar node with 4–6 longitudinal carinae, very close and in the posterior slope joined with circular and regular pattern.

Ecology: *M. morettoi* n. sp. nesting in the ground.

Myrmicaria salambo (Figs. 2, 5, 7, 9). TL 6.2–8.54 mm. Orange-ochre or reddish-ochre in colour. Head and pronotum areolate-reticulated. Clypeus with shortly median carina. Femora subopaque and extensively striated. Petiole with subpetiolar process formed by two teeth always separate apically. Venter of postpetiole with a protrusion anteriorly obtuse (never angled or pointed). Pronotum and mesonotum with median raised carina, remaining carinae linear with evident anastomosis. Dorsum of propodeum with irregular carinae and anastomosis. Nodes of peduncle carinated.

Ecology: *M. salambo* is arboreal and attending climbing insects (Wheeler, 1922).

Concerning the measurements, *M. morettoi* n. sp. has CW, SL, CI and CS relatively much smaller than *M. salambo* (see Table 1). The measures of *M. salambo* are based on 3 syntypes (MCZ) and 4 specimens from Ivory Coast, Boundiali, M'banto 09°35'18.1"N 006°42'52.1"O, piège achatine, Moretto P. leg. (ASPC). This last datum represents the first citation from Ivory Coast and largely expands to the West the geographical distribution of *M. salambo*.

	M. morettoi n. sp. (n. 12) mm (min-max)	*M. salambo* (n. 7) mm (min –max)
CI	97.368 - 105.55	96.770 - 117.50
CL	1.476 - 1.60	1.517 - 1.984
CS	1.5170 - 1.608	1.619 - 1.952
CW	1.504 - 1.616	1.712 - 1.927
FLD	0.656 - 0.779	0.738 - 0.82
GL	1.927 - 2.01	2.132 - 2.46
MH	0.984 - 1.376	1.025 - 1.189
ML	1.517 - 1.804	1.763 - 1.968
PH	0.533 - 0.592	0.533 - 0.615
PL	0.574 - 1.025	1.066 - 1.517
PSD	0.416 - 0.56	0.533 - 0.615
PPH	0.574 - 0.624	0.656 - 0.779
PPL	0.451 - 0.624	0.451 - 0.533
PPSD	1.744 - 2.091	2.050 - 2.132
SI	94.87 - 100	93.47 - 102.38
SL	1.558 - 1.696	1.728 - 1.927
SPSD	0.656 - 0.820	0.779 - 0.943
TL	6.00 - 7.80	6.20 - 8.54

Table 1. Measurements of *Myrmicaria morettoi* n. sp. and *M. salambo* (see text).

Figures 3-5: Ring-like supetiolar process in *M. morettoi* n. sp. (Fig. 3); unarmed petiole in *M. morettoi* n. sp. (Fig. 4); subpetiolar teeth in *M. salambo* (Fig. 5). Figures 6, 7. Head of *M. morettoi* n. sp. (Fig. 6); Head of *M. salambo* (Fig. 7). Figures 8, 9: Mesosoma of M. morettoi n. sp. (Fig. 8); Mesosoma of *M. salambo* (Fig. 9).

I am not sure that the paratypes of *M. morettoi* n. sp. lacking subpetiolar teeth (Fig. 4), come from the same nest as the holotype. However, in respect to the paratypes with armed petiole I did not find any differences in the body sculpture, colour, clypeus etc., and for these reasons I think they are probably all members of the same colony and consequently of the same new taxon.

It is obvious that the presence of the subpetiolar process in *M. morettoi* n. sp. and in *M. salambo* easily distinguishes them from all other *Myrmicaria* (including Asian species). Some doubts raise about the aforementioned specimens of *M. morettoi* n. sp. lacking subpetiolar teeth.

Therefore, I have examined the types of the other Central and Western African species, preserved in the Genoa Museum or photographed in the Antweb.org website. Below my results, where I emphasize the peculiar characters of each species, not present in the new species:

Myrmicaria exigua Andrè, 1890 *sensu stricto* and subspecies: reduced size TL 3–4.5 mm. Antenna not clubbed with only the last funicular segment elongated (in the other African *Myrmicaria* the antenna is clubbed in three segments).

Myrmicaria opaciventris Emery, 1893 *sensu stricto* and subspecies: body orange-ochre in colour. Surface of head and mesosoma opaque, gaster with half part of the first tergite opaque, very rugose or rugged, remaining tergites smooth. Femora well striated. Venter of postpetiole with a protusion anteriorly angled or pointed. Note that in the venter of petiole in the ssp. *congolensis* and ssp. *mesonotalis* it is present a translucent linear lamella well developed (as in *M. morettoi* n. sp. and *M. salambo*).

Myrmicaria baumi Forel, 1901 *sensu stricto*: dark reddish–brown in colour. Lateral and posterior part of head strongly reticulate lacking longitudinal carinae (present only between the frontal carinae). Dorsum of pronotum with transverse carinae. Mesonotum with longitudinal carinae but lacking median raised carina. Dorsum of propodeum with longitudinal carinae.

Myrmicaria baumi occidentalis Santschi, 1920: body castaneous in colour. Head as long as wide with longitudinal carinae but weakly reticulate. Clypeus without median carina. Pronotum and mesonotum with longitudinal carinae, curved near the anterior margin, without anastomosis except in the anterior pronotal margin). In the venter of petiole, a translucent longitudinal lamella is visible. Postpetiolar node with scarce longitudinal carinae and evidently spaced in the posterior slope (in *M. morettoi* n. sp. the carinae in the posterior slope are very close together). Femora well striated. This subspecies is not related with *M. baumi sensu stricto*, but probably actually is a good species near to *M. morettoi* n. sp., (but different in head subsquare, femora and postpetiolar sculpture). Gaster evidently opaque as in *M. opaciventris*.

Myrmicaria distincta Santschi, 1925: head and mesosoma ochre in colour contrasting with the darkish-brown gaster. Pronotum with longitudinal carinae lacking anastomosis. Propodeum with some longitudinal carinae in dorsal view. Nodes of petiole and postpetiole smooth.

Myrmicaria distincta abissinica Santschi, 1925: body brownish–black in colour that contrasts with inferior part of head and pro-mesothorax reddish-ochre. Carinae on pronotum arched. Propodeum with short longitudinal carinae in dorsal view.

Myrmicaria distincta vorax Santschi, 1933: shape as *M. distincta abissinica* but much darker in colour. Carinae of pronotum arched. Propodeum dorsum with carinae and anastomosis in dorsal view.

Myrmicaria fumata Santschi, 1916 *sensu stricto*: body brown in colour. Dorsum of propodeum with longitudinal carinae and anastomosis in dorsal view. Femora well striated.

Myrmicaria fumata linearis Santschi, 1925: body darkish-ochre in colour. Head with numerous linear longitudinal carinae and some short anastomosis. Postpetiole with ventral protrusion angled anteriorly with the angle almost right. Dorsum of propodeum with longitudinal carinae in dorsal view.

Myrmicaria fusca Stitz, 1911: entire body surface smooth and polished. Face of head lacking longitudinal carinae except some short and fine carinae in the vertex, median carina visible between the frontal lobes only. In lateral view, sides of mesosoma and abdominal peduncle lacking longitudinal carinae. Dorsum of mesosoma lacking longitudinal carinae and anastomosis except for the median carina and two short oblique carinae placed at sides of the pronotum in dorsal view.

Myrmicaria striata Stitz, 1911: disc of pronotum with transverse carinae. Mesonotum with longitudinal carinae. Dorsum of propodeum with 1–2 short longitudinal carinae.

Myrmicaria striata buttgenbachi Forel, 1913: body surface smooth and polished; general sculpture with longitudinal carinae regular linear but without anastomosis (except some very short anastomosis in the temples of head). Head with only 2–3 fine longitudinal carinae at sides of median carina. Pronotum with arched carinae, median carina not complete.

Myrmicaria striata insularis Santschi, 1920: head with longitudinal carinae irregular, interrupted, spaced and without anastomosis. Pedicel nodes smooth and shiny. Erect setae fine and yellow.

Myrmicaria irregularis Santschi, 1920: body surface smooth and polished and castaneous in colour. Head with 1–2 short carinae at side of the median carina in the frons area. Mesosoma with median carina and 2 or 3 longitudinal carinae lacking anastomosis. Venter of postpetiole with anterior process angled (similar in this character to *M. opaciventris* and *M. fumata linearis*).

Myrmicaria n. sp. of Senegal mentioned in Ants of Africa.org: body black in colour and much setose, with erect setae short and paler. Second and third funicular segments of the same length (in *M. morettoi* n. sp. they are different in length). Clypeus smooth showing only very faint traces of carinae (as *M. morettoi* n. sp.). Venter of petiole lacking teeth.

In conclusion, I think that *M. baumi occidentalis* is the taxon that more than others can be confused with the specimens of *M. morettoi* n. sp. lacking subpetiolar teeth, but the much spaced carinae present in the posterior side of postpetiole let immediately distinguish it.

ACKNOWLEDGMENTS

I am gratefully to following peoples: L. Toledano (MSNV) for a linguistic revision of the text; R. Poggi (MSNG) and S. Cover (MCZ Harvard University) for the loans of type material, P. Moretto (Toulon, France) collector of African ants in the field, and at last the anonymous referees.

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Printed in Palermo by
Edizioni Danaus
Date of publication: 30.12.2019

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Please provide the first page according to the following scheme:
- Title of the paper (not capitalised);
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Manuscript should be assembled in the following order: Introduction, Materials and Methods, Results, Discussion, Conclusion (if applicable), Acknowledgements (optional), References. Tables and figures (JPEG/TIFF format – 300 DPI) should be cited in the appropriate area in the text and numbered consecutively.

Bibliographic references in the text must be given as follows:
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